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## The role of population pharmacokinetic-pharmacodynamic modelling in antimalarial chemotherapy

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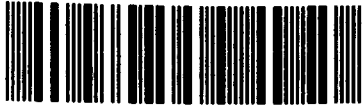
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**THE ROLE OF POPULATION PHARMACOKINETIC-  
PHARMACODYNAMIC MODELLING IN ANTIMALARIAL  
CHEMOTHERAPY**

JULIE ANNE SIMPSON, BSc, Diploma of Mathematical Statistics

A thesis submitted in partial fulfilment of the requirements of the Open University for the  
degree of Doctor of Philosophy

May 2001

Wellcome Trust Research Unit, Bangkok

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## **Declaration**

This dissertation describes my original work except where acknowledgement is made in the text. It does not exceed the page limit and is not substantially the same as any work that has been, or is being, submitted to any other university for any degree, diploma or any other qualification.

Julie Anne Simpson, May 2001

## Dedication

This thesis is dedicated to my husband Hugh, my parents, Annette and Bob, and my grandmothers, Hazel and Helen. All of you have given me years of love, support, encouragement and laughter.

*Mathematics is not a careful march down a well-cleared highway, but a journey into a strange wilderness, where the explorers often get lost.*

*W.S. Anglin*

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## List of Abbreviations

AUC <sub>0-∞</sub>	- Area under concentration-time curve
CI	- Confidence interval
CL/F	- Apparent clearance
GTS	- Global two-stage method
HPLC	- High performance liquid chromatography
MIC	- Minimum Inhibitory Concentration
MPC	- Minimal Parasitocidal Concentration
NLME	- Nonlinear mixed-effects
PCT	- Parasite clearance time
PC <sub>50</sub>	- Time to 50% reduction in parasitaemia
PC <sub>90</sub>	- Time to 90% reduction in parasitaemia
PD	- Pharmacodynamics
PI	- Prediction interval
PK	- Pharmacokinetics
PMR	- Parasite Multiplication Rate
PRR	- Parasite Reduction Ratio
SD	- Standard deviation
SE	- Standard error
STS	- Standard two-stage method
V/F	- Apparent volume of distribution

## **Layout of thesis**

The layout of this thesis differs from standard layouts in order to make it easier to follow and read.

The first chapter is a general introduction.

The second chapter details the aims and objectives of the study.

The next four chapters, chapters 3, 4, 5 and 6, relate to four distinct phases of work, as indicated by the four aims of the thesis. These chapters will discuss each of the four phases of work in turn. Each chapter will consist of an introduction, methods, results and discussion.

The final chapter, chapter 7, is a general discussion with implications for future research.



## ABSTRACT

Despite the high rates of mortality and morbidity associated with malaria very few new antimalarial drugs are being developed. Hence there is much to be gained by optimising currently available antimalarials.

Using pharmacokinetic and pharmacodynamic data from field trials where mefloquine was administered either alone or in combination with artesunate important information regarding optimal dosing for the clinician has been obtained. The pharmacokinetics of mefloquine were influenced relatively little, or not at all, by factors such as age, sex, or measures of acute malaria severity. Splitting the 25 mg/kg dose of mefloquine monotherapy was associated with a 50% increase in oral bioavailability, and bioavailability increased by 20% for the split dose on the combination treatment. Oral bioavailability was also observed to be associated with more rapid parasite clearance, a surrogate outcome measure for cure.

A mechanistic mathematical model was developed that describes the change in total parasite burden with time *in vivo*, in the presence of mefloquine. Estimates for the pharmacokinetic and pharmacodynamic parameters were obtained from population modelling of pharmacokinetic and *in vitro* pharmacodynamic data, both derived from the same region. Model simulations confirmed that, early in the evolution of resistance, conventional assessments of the therapeutic response 28 days after treatment underestimate considerably the level of resistance. Longer follow-up is required. The model also indicates that initial deployment of a lower (15-mg/kg) dose of mefloquine provides a greater opportunity for the selection of resistant mutants and would be expected to lead more rapidly to resistance than *de novo* use of the higher (25-mg/kg) dose.

From the modelling of malaria therapy data it was observed that even in the absence of drugs the patient parasitaemia versus time profiles varied greatly between both patients and parasites. Parasite multiplication rate every 48 hours was estimated to be approximately 8 fold.

# 1. INTRODUCTION

Each year approximately 270 million people suffer from malaria with an annual mortality of somewhere between 1 and 2.5 million deaths (World Health Organisation 1998). The development of new medicines for treating malaria is essential since malaria parasites have acquired resistance to nearly all established antimalarial drugs (Winstanley, 2000). Despite the urgent need for new therapeutic agents there is little financial gain for drug companies to produce drugs that are mainly needed for developing countries where malaria is predominate. Of the 1223 new molecular entities sold worldwide during 1975-96, less than 1% were destined for tropical diseases (Trouiller and Olliaro, 1999). With very few new drugs for malaria there is much to be gained by optimising currently available drug therapies.

Malaria is a disease caused by the invasion of the red blood cells by the *Plasmodium* parasites. *Plasmodium falciparum*, one of the four species of human malaria, is the most dangerous species causing almost all severe disease and deaths. Despite attempts to eradicate malaria the disease is still endemic in many parts of the world. One of the major contributing factors to its continued presence is the emergence of malaria parasites which are resistant to one or more of the antimalarial drugs. Drug resistance is defined as the 'ability of a parasite to multiply or to survive in the presence of concentrations of a drug that would normally destroy parasites of the same species or prevent their multiplication' (World Health Organisation 1973). Drug resistance arises as a result of genetic mutations that reduce the parasite susceptibility to antimalarial drugs.

Studies monitoring the therapeutic efficacy of drugs *in vivo* and the minimum inhibitory concentrations (MIC) of the drug *in vitro* need to be reported regularly in order to detect

emerging drug resistance as soon as possible. Pharmacokinetic studies investigate the changes in drug concentration over time and the association between the pharmacokinetic parameters (e.g. absorption rate of the drug) and the dynamics of the disease. These studies may aid the doctor in determining the most beneficial way to give the drug.

The pharmacokinetic properties of antimalarial drugs have been studied extensively using the conventional approach of multiple sampling in relatively few healthy volunteers or patients. However there is an increasing interest in the design of population based studies using a much larger number of subjects with fewer samples (often 2-4) collected at random times. These studies may provide information that is more representative of, and thus relevant to, the patient population of interest since they allow characterisation of the different sources of variation in the pharmacokinetic parameters. Population modelling approaches seek to: relate dose to effect, explain some of the inter-patient variability with covariate analysis, and characterise unexplained inter-individual variability. Some examples of population modelling of studies where only sparse data has been used to generate population models of pharmacokinetics and pharmacodynamics are theophylline used in the treatment of asthma (Driscoll et al. 1989) and anti-HIV treatment with zidovudine (Gitterman et al. 1990).

In some areas mefloquine is the treatment of choice for uncomplicated multi-drug resistant falciparum malaria. Little work has been done using the population approach to investigate how mefloquine concentrations relate to the therapeutic effect. Large inter-individual variability has been reported for mefloquine indicating that population pharmacokinetic-pharmacodynamic modelling is needed to provide a framework for optimisation of mefloquine dosing regimens.

On the Thai-Burmese border pharmacokinetic data were collected prospectively from patients receiving mefloquine as part of trials conducted between 1990 and 1995. Prior to 1994 mefloquine was given alone and thereafter in combination with artesunate. Using these data the population pharmacokinetic parameters for mefloquine were estimated. In order to derive clinically useful dosing guidelines pharmacokinetic models must be combined with pharmacodynamic models. Population pharmacodynamic models of *in vivo* mefloquine actions were developed using a combination of *in vitro* pharmacodynamic data, population parasitaemia time series in untreated patients (available historical data) and from the data mentioned above. After the above models were defined the relationship between these models of kinetics and dynamics were determined using sequential pharmacokinetic-pharmacodynamic modelling (Davidian and Giltinan, 1995).

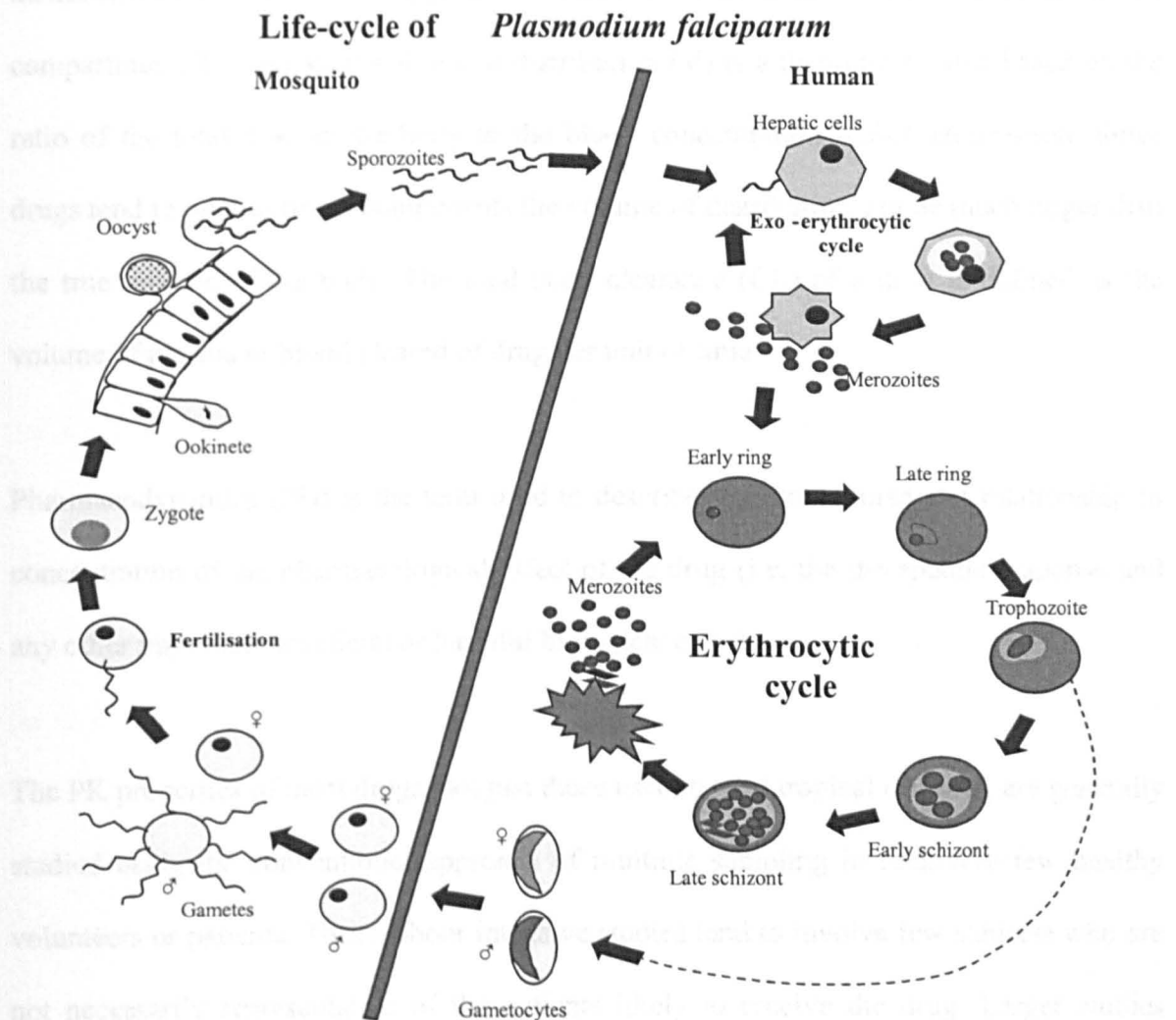
Ultimately the population modelling approaches that were applied to mefloquine in this thesis will highlight the factors influencing inter-patient variability in the dose-response relationship, and provide the clinician with optimal dosing strategies. The new approach rationalising the use of pharmacokinetic analysis in relation to the efficacy of mefloquine presented here may also be used as a guideline for optimisation of other antimalarial drugs.

## **1.1 Malaria**

Malaria is a disease caused by the invasion of the red blood cells by the *Plasmodium* parasite. Transmission of malaria to humans results from the bite of an infected female *Anopheles* mosquito. There are four species of *Plasmodium* infecting humans, *falciparum*, *vivax*, *ovale* and *malariae*. *Plasmodium falciparum* is the most dangerous species causing almost all severe disease and deaths of human malaria.

The life cycle of *Plasmodium falciparum* is described below and illustrated in Figure 1.1:-

- a) The *P. falciparum* parasite is injected into the blood stream of the human host usually by the infected female *Anopheles* mosquito in the form of sporozoites.
- b) The sporozoites invade the liver after a few minutes.
- c) In the liver the sporozoite multiplies over a period of 5-7 days, the pre-erythrocytic incubation period.
- d) After development in the liver the hepatic schizonts rupture to release merozoites into the bloodstream. The merozoites enter the red blood cells where they feed on the contents of the cells. This begins the intra-erythrocytic asexual cycle that causes the disease malaria. The parasite first develops as a tiny, then small, and then large ring. The parasite begins to digest haemoglobin to produce haemazoin pigment, which is deposited within the food vacuole. At this stage the parasite is called a mature trophozoite.
- e) After 18-26 hours (approximately when pigment is first visible microscopically), the trophozoites cause the red blood cells to adhere to the endothelium of the small blood vessels. In this period (26-48 hours), these older stages of the parasite sequester in the vasculature of deep tissues and are not readily seen in the peripheral blood film.
- f) The trophozoite divides into daughter merozoites to become a schizont which then ruptures releasing 16-32 merozoites into the blood ready to infect other red blood cells. This whole process takes approximately 48 hours.
- g) Merozoites that invade the red blood cells can take one of two development routes: either develop into an asexual schizont and multiply further or become a gametocyte (the sexual stage of the parasite). The gametocyte develops over several days. When another anopheline mosquito bites the human host, the mature gametocyte can be ingested in the blood meal and infect the mosquito, where sexual reproduction (meiosis) occurs and the cycle begins again.



**Figure 1.1** The life cycle of *Plasmodium falciparum*

## 1.2 Population pharmacokinetic and pharmacodynamic modelling

Pharmacokinetics (PK) is the study of the relationship between the dose of a drug and the manner in which its concentrations in the body change over time. A PK model provides a mathematical representation of the processes by which the drug enters the body (absorption), moves around the body (distribution) and leaves the body (elimination). The body is considered to consist of one or more “compartments”. In a one compartment model, the drug enters the compartment either instantaneously (intravenous bolus

injection) or gradually (e.g. infusion, intramuscular or oral administration). The drug then distributes evenly to fill an “apparent” volume of distribution, and is cleared from this compartment. The apparent volume of distribution (V) is a theoretical value based on the ratio of the total dose in the body to the blood concentration before elimination. Since drugs tend to bind to tissue components the volume of distribution can be much larger than the true volume of the body. The total body clearance (CL) of a drug is defined as the volume of plasma or blood cleared of drug per unit of time.

Pharmacodynamics (PD) is the term used to describe the time course and relationship to concentration of the pharmacological effect of the drug (i.e. the therapeutic response and any other important beneficial or harmful biological effects).

The PK properties of most drugs, not just those used to treat tropical diseases, are generally studied using the conventional approach of multiple sampling in relatively few healthy volunteers or patients. These labour intensive studies tend to involve few subjects who are not necessarily representative of the patients likely to receive the drug. Larger studies carried out on the target population therefore have major advantages (Aarons, 1992). Patient characteristics (e.g. gender, ethnicity, weight, disease severity, hepatic or renal impairment) that might modify the PK behaviour of the drug can be explored and dissected. However intensive blood sampling of a large sample of the target population is not always feasible. It might be medically hazardous for some patients to be sampled repeatedly (e.g. very ill patients, small infants and elderly) or logistically impossible. There are also cost implications particularly for field trials in the developing world, where the main burden of tropical diseases resides.

An alternative to conventional PK studies is now available. This uses PK data derived from only a few blood samples or as little as one per subject, and can be analysed using

population methods (Aarons, 1993). The population approach is based on relatively recent statistical methodology (Vozech et al. 1996). It seeks to estimate the mean PK parameters (e.g. clearance, half-life) and to identify the patient or disease characteristics (i.e. physiological, pathological, environmental or genetic factors) that explain as much as possible of the inter-patient variability of the PK parameters.

### 1.2.1 Pharmacokinetics

#### 1.2.1.1 Traditional pharmacokinetic methods

A drug development program usually consists of three phases. Phase I is a clinical trial to determine the PK behaviour of the drug within a small group of healthy volunteers. Phase II determines the PK behaviour in a small group of patients with the condition intended to be treated. Phase III trials are usually large-scale clinical trials to evaluate safety and efficacy. Data collected during Phase I and II trials have historically been used to determine the PK profiles of a drug. The data consists of frequent sampling from a handful of subjects, not always representative of the patients likely to receive the drug.

The data are traditionally analysed using the standard two-stage approach. The first stage is to estimate the PK parameters for each individual separately. The second stage is to calculate the appropriate centre of the distribution (i.e. mean, median or geometric mean) and variation of the individual PK parameter estimates to obtain estimates of the overall population PK parameters. The obvious advantage of this approach is the simplicity in calculation of the PK parameters.

The disadvantages however are:-

- Numerous blood samples per subject are required.



- This may be difficult for studies conducted on the young, elderly or seriously ill or those with poor venous access. For example the majority of the African population with severe malaria are young anaemic children.
  - This restriction means that subjects with poor compliance will need to be excluded from analysis, thus reducing numbers and possibly introducing bias.
  - Laboratory errors or samples that have been misplaced or stored incorrectly will lead to exclusion of a subject and thus reduce numbers. In many situations in the tropics drug analysis is performed by overseas collaborators (Winstanley and Watkins, 1992); and consequently frozen samples may be lost or ruined during transportation.
  - The difficulties and discomfort involved in serial sampling and the cost of analysis tends to limit the total number of subjects examined that may lead to an unrepresentative sample of the population being studied.
- This approach systematically overestimates the inter-individual variability and therefore is biased (Steimer et al. 1984). Each individual PK parameter is estimated from the original drug concentration-time data with some error. This error adds variability to the PK parameter estimates thus increasing the estimate of the inter-individual variability.
  - All data from each individual must be described by the same PK model. If fifty percent of the subjects are fitted with a two-compartment model and the remaining subjects are fitted with a one-compartment model then it is not possible to combine the four parameters from the two-compartment model with the two parameters from the one-compartment model.

### 1.2.1.2 Population pharmacokinetic methods

In contrast to traditional PK methods, the population approach to analyse PK data requires only a few blood samples collected from each patient. As fewer samples are required per patient in a population study, it is feasible to study a greater number of patients and also to study patients where it may not be ethical or logistically possible to perform intensive serial blood sampling. For example, a population PK study could be incorporated into a Phase III clinical trial. The patient population in Phase III clinical trials, is more representative of the overall population for which the drug will be finally used (Sheiner and Benet, 1985).

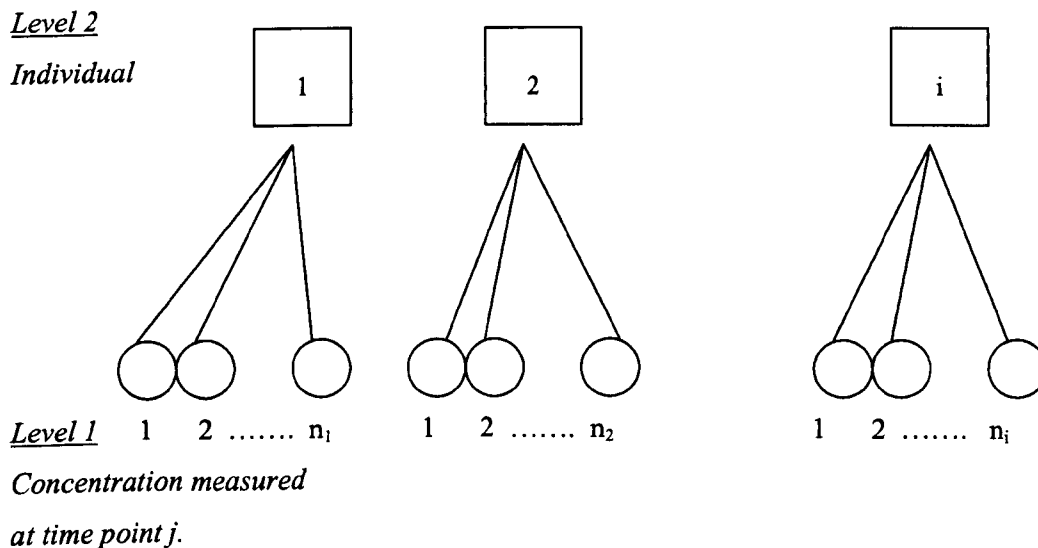
The advantages of the population approach are:-

- All patients are included in the analysis. Patients where a blood sample was misplaced or not recorded are not excluded.
- Samples do not need to be collected from subjects at the same times. In fact variation in the time of collection is often desirable.
- For the same cost more subjects can be studied since fewer samples per subject are required.
- Accurate estimates of the inter-individual, intra-individual, and inter-occasion variability are feasible.
- Sub-groups of patients that are different from the general population can be identified and consequently information on dosing requirements obtained.
- The association between the therapeutic response and PK of a drug can be explored to determine risk:benefit relationships.
- Posterior individual PK parameter estimates can be obtained from the population analysis.

The disadvantages of the population approach are:-

- Certain preliminary PK information should be known before any population PK study is undertaken.
- Logistically it is more complicated compared with traditional PK methods.
- It requires statistical expertise.
- It is a computer-intensive technique.
- Occasionally the sparse data collected during a population PK study may not provide adequate information for the characterisation of a reasonable PK model.

The population approach for analysis of PK data is termed hierarchical nonlinear mixed effects modelling. The term hierarchical is used to describe the data structure. Generally there are two levels in PK data: the measured concentration at the  $j^{\text{th}}$  time (level 1) of the  $i^{\text{th}}$  individual (level 2) (see Figure 1.2 below).



**Figure 1.2:-** Pharmacokinetic data structure for the two-level hierarchical model.

The nonlinear mixed effects model can be described by the following equation:-

$$C_{ij} = f(x_{ij}, \beta_i) + \varepsilon_{ij}$$

- $C_{ij}$  represents the  $j^{\text{th}}$  concentration of the  $i^{\text{th}}$  individual.
- $f(x_{ij}, \beta_i)$  denotes a nonlinear function of some independent variables,  $x_{ij}$  (e.g. time, dose, etc..) and some PK parameters,  $\beta_i$  (e.g. clearance for the  $i^{\text{th}}$  individual).
- $\varepsilon_{ij}$  represents the residual error (also known as intra-individual error), that is the difference between the predicted and observed values, and is assumed to have zero mean and an unknown variance ( $\sigma^2_\varepsilon$ ).

The  $f(x_{ij}, \beta_i)$  comprises of two parts: a structural model (this is usually a conventional PK model, e.g. a one-compartment model, which may also involve covariates such as weight), and a variance model. The variance model describes the difference between the drug concentration of an individual and that predicted by the structural model. The parameters,  $\beta_i$  (e.g. absorption rate), vary across individuals and can be modelled using the following equation:-

$$\beta_i = \beta + b_i$$

where  $\beta$  is a vector of fixed effects (the mean population PK parameters and may also include individual covariates) and  $b_i$  is a vector of random effects. The  $b_i$  are assumed

to arise from a population distribution with zero mean and a covariance matrix,  $D$ , which quantifies the random inter-individual variation.

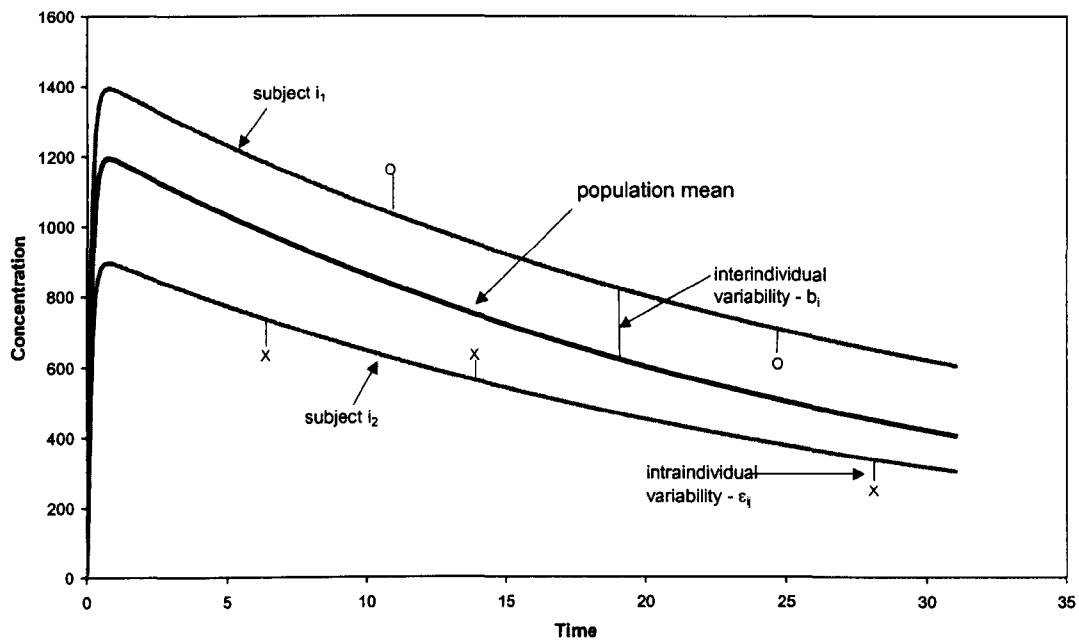
The data for all individuals are analysed simultaneously, with the mixed effects model taking into account both the fixed and random effects (described below).

- Fixed effects parameters.

These may be the mean values of the PK parameters or a number of parameters that relate the PK parameters to demographic and pathophysiological variables, such as age, body weight, blood pressure, etc.

- Random effects parameters.

There are two types. The variances of the PK parameters (inter-individual variability within the population) and the residual intra-individual variability due to random fluctuations in an individual's parameter values, measurement error and all other sources of error not accounted for by the other parameters. These two sources of variability are contrasted in Figure 1.3.



**Figure 1.3:-** Sources of population PK variability. The black solid line represents the population mean profile. The grey solid lines represent the individual profiles for subject  $i_1$  (data O) and subject  $i_2$  (data X). The difference between an individual profile and the population mean profile is due to inter-individual variability while the difference between the observed data and the individual profile is due to intra-individual variability.

The computer programmes that are capable of analysing data using hierarchical modelling are not simple to use and the user needs to be fairly familiar with the philosophy and theory of population PK. For a detailed review of the programmes available see Aarons (1999) and Davidian and Giltinan (1995). Maximum likelihood is the main estimation method used for hierarchical modelling. Maximum likelihood methods are implemented by NONMEM (a procedure developed in 1979 (Beal and Sheiner, 1980)) and the NLME procedure (Lindstrom and Bates, 1990) of the statistical computing package, S-PLUS (StatSci, Mathsoft Inc. Seattle, Washington). Other methods available for the analysis of population PK data include nonparametric methods (Mallet, 1986; Steimer et al. 1984) and Bayesian methods (Best et al. 1995; Spiegelhalter et al. 1997).

### 1.2.1.3 Design of population pharmacokinetic studies

The design of population PK studies is a relatively new area of research and precise methodology has yet to be defined. Population PK studies can form part of large clinical trials (usually Phase III trials) where a few blood samples are taken from each patient or from a randomly selected sub-group of patients. This extra data collection should not alter greatly the original protocol or cause ethical problems (Aarons et al. 1996).

To determine the timing of blood samples computer simulation and optimal design methods (D'Argenio, 1981; Mentré et al. 1997; Retout et al. 2001) can be used. These methods ensure that data are collected at informative times for estimation of PK parameters. The study investigator is given a sampling window (that is, a range of times rather than a particular time) when a blood sample must be collected. Patients should be sampled throughout the duration of the study to allow for changes over time in the PK parameters (known as inter-occasion variability).

The protocol for population PK studies should not be overly complicated, and the times of blood sampling should be convenient both to patients and to clinical staff. The investigators and patients should understand the necessity of blood sampling and the importance of accurate recording of the time the blood sample was taken. Sun et al. (1999) provide more detailed recommendations regarding the design of population PK studies.

Population PK studies allow a great deal of freedom concerning the inclusion criteria and the blood sample collection. The studies require fewer design criteria than other methods and are adaptable to the clinical setting. However there are still practical issues to consider, such as integration of the population PK study within the existing clinical trial protocol, quality control of the data, and the extra time, cost and expertise required for laboratory and statistical analyses.

### 1.2.2 Pharmacodynamics

The principles discussed above are also applicable to PD data. A common modelling approach is to relate a single measure of drug exposure (e.g. area under the concentration-time curve) to a single measure of drug effect (e.g. time to maximum effect). More difficult approaches relate the entire time course of drug concentration to the time course of drug effect. Additional factors that may have to be modelled are disease progression and drug interactions.

*In vivo* the PD properties of antimalarial drugs are assessed by clinical outcome (mortality, speed of recovery from coma and fever clearance) and parasitological outcome (White and Krishna, 1989). Parasitological recovery from malaria is measured by the clearance of parasites from peripheral blood smears. The measures currently used are parasite clearance time (PCT; which is the time from initiation of treatment to the first of at least two negative smears), PC<sub>50</sub> (time until parasitaemia has been reduced by 50%), PC<sub>90</sub> (time until parasitaemia has been reduced by 90%) and PRR<sub>48</sub> (parasite reduction ratio at 48 hours) (White, 1997). Unfortunately the parameters above do not describe the time course of the effect and therefore might not be adequate for describing the efficacy of the drug in the host since the amount of drug available changes over time. Additionally there are methodological and scientific limitations associated with measuring the parasite burden from peripheral blood smears. The limit of detection for microscopy is around 50 parasites / $\mu$ l, therefore adult patients with less than  $2.5 \times 10^8$  parasites in the blood stream will be classified as aparasitaemic. Furthermore the relationship between the number of parasites detected by the microscope and disease severity is not straightforward. A patient may be in a deep coma yet have only a few parasites visible by microscopy whereas a child may be able to walk and eat when they have much higher parasite burdens. The explanation for these phenomena is that for *P. falciparum* malaria only the first half of the 48 hour life



cycle of the parasite is visible to the microscopist (explained in more detail in section 1.1). Thus more representative measures of the PD properties of antimalarial drugs *in vivo* are needed to assess accurately and predict the therapeutic response of the antimalarial drugs.

### 1.2.3 Pharmacokinetic-Pharmacodynamic modelling

In the past the relationship between drug dosage and therapeutic response was investigated by randomising patients to one of several dose groups and comparing the response profiles. The problem with this method is that the dose response profile may be blurred by substantial inter-patient variation in drug kinetics. This can now be overcome by mathematical modelling of the kinetics and dynamics together. There are two approaches to this type of modelling, that is, sequential or simultaneous modelling.

#### 1.2.3.1 Sequential modelling

Firstly the PK model is fitted to the concentration-time data. The individual specific PK parameter estimates obtained are then used to predict concentrations corresponding to the times at which PD measurements were taken.

For example:- the  $E_{\max}$  model,

$$y_{ij} = E_{0i} + \left( \frac{E_{\max i} - E_{0i}}{1 + \left( \frac{EC_{50i}}{C_{ij}} \right)} \right) + e_{ij}$$

$e_{ij}$  – within subject error

$C_{ij}$  – drug concentration at  $j^{\text{th}}$  measurement for the  $i^{\text{th}}$  subject

The models can include inter-individual error on the dynamic parameters  $E_{0i}$ ,  $E_{\max i}$  &  $EC_{50i}$ .

### 1.2.3.2 Simultaneous modelling

Fitting both the PK and PD models together. This allows simultaneous assessment of the PK and PD variability.

$n_i$  - total number of PK and PD measurements on subject  $i$ .

Vector  $y_i$  [size- $n_i \times 1$ ] - denotes stacked PK and PD measurements taken on subject  $i$ .

$C_p(t_{ij})$  - PK model (e.g. one or two compartment models)

$$y_{ij} = E_{0i} + \left( \frac{E_{\max i} - E_{0i}}{1 + \left( \frac{EC_{50i}}{C_p(t_{ij})} \right)} \right) + e_{ij}$$

The models can include inter-individual variation on both the PK and PD parameters.

### 1.2.3.3 Problems associated with pharmacokinetic-pharmacodynamic modelling

- a) To model successfully the response a reasonable range of concentrations across subjects is required.
- b) It is important to choose the appropriate response for the PD model. In other words the clinical relevance, ease of measurement, variability in the response and overall sensitivity to changes in drug concentration need to be considered.
- c) For some effect measures there is a possible lag time between the plasma concentration and PD effect. This occurs when the PK measurements are not taken directly at the effect site. To investigate this plot both the effect and plasma concentrations versus time. This plot is called a hysteresis curve. The distance between curves at different times reflects the degree of hysteresis. There are two ways to deal with varying degrees of hysteresis: only use plasma concentrations at steady state where the plasma concentration and effect levels are a constant ratio or

extend the PK model to include a hypothetical 'effect' compartment. In the case of antimalarial efficacy the "effect compartment" is the intra-erythrocytic parasite.

- d) If the measurement error of drug concentrations is ignored then a possible bias could affect the PD parameters.

There is a lack of consensus in the literature about whether it is more informative to perform a sequential or simultaneous PK-PD analysis, however it is well established that the presence of PK data improves the precision of the estimates of the PD parameter values (Hashimoto and Sheiner, 1991).

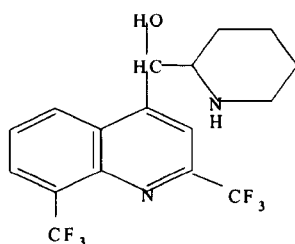
### **1.3 Mefloquine**

Mefloquine was introduced in Thailand at the end of 1984 as the first line treatment for falciparum malaria. It was developed in the 1960s as part of a programme by the Walter Reed Army Institute of Research, Washington, USA, to identify and develop antimalarial drugs effective against resistant strains of *P. falciparum*. Mefloquine is effective for all species of malaria parasites infecting humans, including multidrug-resistant *P. falciparum*. It is given both as a prophylactic and therapeutic agent (Palmer et al. 1993).

In some areas mefloquine, in combination with an artemisinin derivative, is the only efficacious treatment left for uncomplicated highly multi-drug resistant *P. falciparum* malaria.

#### **1.3.1 Structure and activity**

Mefloquine is a quinoline-methanol antimalarial, structurally related to quinine (Figure 1.4).



**Figure 1.4** Structural formula of mefloquine

Mefloquine has two enantiomers: the (+) RS-enantiomer and (-) SR-enantiomer. An enantiomer is a compound derived from a molecule that exhibits optical-isomerism because of the presence of one or more optically active chemical centres.

This agent is active against the asexual stages of parasite development (see section 1.1 above), but has no activity against mature gametocytes or against the sporozoites. During the asexual stage, mefloquine has greater inhibitory activity against the more mature trophozoite and schizont forms than against the younger ring stages (Geary et al. 1989).

Despite knowledge of when mefloquine inhibitory activity occurs the precise mode of action of mefloquine remains obscure. Three primary mechanisms of antimalarial activity have been postulated:

- 1) Drugs that are active against the mature stages of the parasite are proposed to accumulate within the food vacuole of the parasite. Haem (ferriprotoporphyrin IX) produced from the parasite digestion of the host cells' haemoglobin is also found within the food vacuole. Haem can either bind with the drug or with protein to form

haemozoin (a malaria pigment not harmful for the parasite). Unlike haemozoin, the drug-haem complex is toxic to malaria parasites (Fitch, 1983; Warhurst, 1987).

- 2) Mefloquine adheres to the DNA of the parasite and thus affects transcription.
- 3) The parasite is unable to digest host cell haemoglobin because mefloquine reduces the acidity of the parasite food vacuole (known as vacuolar alkalinization). This hypothesis is weak since Ginsburg et al. (1989) found no correlation between the antimalarial activity of the drugs and pH levels within the parasite food vacuole.

Of the above three mechanisms it is believed that mechanism one, the inhibition of binding between haem and protein, is the most plausible. However it should be noted that this mechanism, which has been shown for chloroquine (Slater and Cerami, 1992), is still unproven for mefloquine.

### 1.3.2 Formulations

Mefloquine is an antimalarial drug administered orally. There is no intravenous formulation as parenteral administration is associated with local irritation (Goldsmith, 1989). Thus determination of the absolute oral bioavailability of mefloquine is not possible.

### 1.3.3 Pharmacokinetic properties

Several assay methodologies have been developed to measure mefloquine concentration levels. High performance liquid chromatography is the most frequently used (Edstein et al. 1991; Green et al. 1999). The concentration levels of the (+) RS- and (-) SR- enantiomers of mefloquine can also be measured by high performance liquid chromatography (Bergqvist et al. 1994).

After oral administration mefloquine is absorbed, and then extensively distributed in the tissues before being cleared slowly from the body. The PK profile of mefloquine is usually modelled using a two-compartment model, sometimes even a three-compartment model. Studies have shown the half life of the drug to be between 14 and 28 days; apparent volume of distribution (V/F) to range between 13 and 41 l/kg; and apparent oral clearance (CL/F) to lie within 0.02 up to 0.07 l/kg/h (Karbwang and White, 1990).

Considerable inter-individual variation in the PK parameters of mefloquine has been reported (Karbwang and White, 1990) yet few factors have been shown to alter the pharmacokinetics. Higher blood drug concentrations (approximately double) have been reported in adult male Thai patients with acute falciparum malaria compared to healthy adult male Causcasian volunteers (Looareesuwan et al. 1987), these could be due to either genetic or disease related factors. Other studies comparing malaria patients and healthy volunteers within the same ethnic group have conflicting findings (Karbwang et al. 1988; Juma and Ogeto, 1989; Boudreau et al. 1990). Karbwang et al. (1988) found reduced elimination half-life and mean residence time in Thai patients compared to healthy Thai volunteers. In contrast, Juma and Ogeto (1989) found a longer elimination half-life together with reduced total clearance and volume of distribution for severe falciparum malaria in Kenyan patients compared to healthy volunteers. Boudreau et al. (1990) demonstrated a higher plasma  $C_{max}$  and prolonged  $t_{max}$  in Thai patients compared with healthy Thai volunteers. Food has been observed to alter the absorption kinetics of mefloquine (Crevoisier et al. 1997). Peak plasma concentrations of mefloquine were achieved earlier and were higher in the non-fasting state. These changes in absorption resulted in an increased bioavailability of mefloquine of 40% and thus suggesting mefloquine should be administered at meal times. Mefloquine bioavailability was also found to increase for patients receiving mefloquine (as part of the combination therapy:

artesunate plus mefloquine) during convalescence as opposed to the acute stage of disease (Price et al. 1999a).

There is good agreement between mefloquine levels measured in the whole blood of the capillary and the vein (ter Kuile et al. 1994). Venous levels of mefloquine are 1.15 (95% CI: 1.03 – 1.29) times greater than levels measured in the plasma (ter Kuile et al. 1994). However, Daniel Todd et al. (1997) found mefloquine levels measured in the serum to be 1.28 (95% CI: 1.24 – 1.32) times larger than the levels measured in the whole blood.

The plasma protein binding for mefloquine has been reported to be approximately 98% (White, 1992c). However it should be noted that it is difficult to determine the plasma protein binding accurately since mefloquine *in vitro* sticks avidly to plastics.

#### 1.3.4 Pharmacodynamic properties

Mefloquine appears to have relatively little effect on asexual malaria parasites in the first half (24 hrs) of the life cycle (White, 1997) and exhibits its greatest inhibitory activity against the more mature trophozoite and schizont forms (Geary et al. 1989). As a result most circulating *P. falciparum* parasites continue to mature in the host and sequester.

Following treatment with mefloquine, the average parasitaemia-time profile is that of a lag phase, during which the population mean parasitaemia remains unchanged before beginning to decline. Mefloquine has antimalarial activity comparable to other quinolines, reducing the number of parasites in the body by 100 to 1000 fold per parasite life cycle (White, 1997). The long half-life of mefloquine allows therapeutic concentrations to remain until all the parasites in drug-sensitive infections have been removed.

### 1.3.5 History of therapeutic efficacy

Mefloquine was first introduced in Thailand in 1984, originally in combination with sulphadoxine-pyrimethamine in a single tablet form (MSP, Fansimef®: Roche Pharmaceuticals) in the hope this would preserve its antimalarial activity. However *P. falciparum* in Thailand was already highly resistant to sulphadoxine and pyrimethamine and by 1990 efficacy of this combination tablet had fallen from an initial cure rate of >99% to a cure rate of 71% (Nosten et al. 1991b). Since the combination regimen was no longer superior to mefloquine alone, mefloquine monotherapy was adopted, first at 15 mg/kg and then at 25 mg/kg. This increased dose improved cure rates to 91% (ter Kuile et al. 1992) initially, but by 1994 resistance had worsened further and the cure rate for the 25 mg/kg dose had dropped to 60% (Price et al. 1995). Mefloquine monotherapy for uncomplicated falciparum malaria was discontinued and replaced by a combination of mefloquine (25 mg/kg) and 3 days of artesunate administration (4 mg/kg/day) (Nosten et al. 1994).

### 1.3.6 Present function of mefloquine

Mefloquine is currently prescribed as prophylaxis for malaria for people travelling to endemic areas with chloroquine resistant malaria. With regards to the treatment, mefloquine is only used for the treatment of uncomplicated malarial infections. Mefloquine is not a suitable drug for the treatment of severe malaria since it can only be given orally and is associated with a high rate of vomiting. In areas where the level of drug resistance is high, such as in Southeast Asia, mefloquine is now given as part of a combination therapy (mefloquine plus an artemisinin derivative) for the treatment of uncomplicated *P. falciparum* infections (Nosten et al. 2000; Price, 2000; Nosten et al. 1994). Mefloquine is too expensive for general use throughout tropical Africa (Winstanley, 2000).



## 2. AIMS AND OBJECTIVES OF THESIS

### 2.1 Aims

- a) To apply population modelling approaches to mefloquine PK data to characterise the factors influencing inter-patient variability in the dose-response relationship, and provide the clinician with optimal dosing strategies.
- b) To develop a mechanistic PK-PD model for mefloquine *in vivo* that can be used to simulate the PD response *in vivo* with different dosing regimes or in areas of varying levels of mefloquine resistance.
- c) To determine the population dynamics of the *Plasmodium falciparum* parasite within the human host in the absence of antimalarial drugs, in order to understand and characterise any subsequent drug effects.
- d) To determine the population *in vivo* PD parameters for mefloquine using sequential PK-PD modelling.

### 2.2 Objectives

#### Aim 1 (Chapter 3)

- a) To estimate the population PK parameters and their inter-individual variability for different mefloquine dosing regimes:-

Mefloquine monotherapy – single dosing (25 mg/kg)

Mefloquine monotherapy – split dosing (15 mg/kg initially, 10 mg/kg 24 hours later)

Mefloquine in combination with artesunate – single dosing of mefloquine (25 mg/kg)

Mefloquine in combination with artesunate - split dosing of mefloquine

(15 mg/kg initially, 10 mg/kg 24 hours later)

- b) To explore the demographic characteristics and disease related factors that influence the PK parameters.
- c) To investigate the relationship between the individual PK parameters and therapeutic response where therapeutic response was measured by parasite and fever clearance and treatment failure.

#### Aim 2 (Chapter 4)

- a) To develop a mechanistic mathematical equation that describes the change in total parasite burden over time *in vivo* in the presence of the antimalarial, mefloquine.
- b) To estimate the population PD parameters (slope of the concentration-effect curve and the mefloquine concentration that is 90% effective) and their inter-strain variability from *in vitro* concentration-effect data.
- c) To simulate total parasite burden-time profiles for the following scenarios; different dosages of mefloquine, different slopes of concentration-effect curves, differing levels of mefloquine resistance, and different proportions of free drug available *in vivo*.
- d) To predict the time to recrudescence for an infection that is classified as type RI resistance for different killing actions of mefloquine and differing levels of mefloquine resistance.

### Aim 3 (Chapter 5)

- a) To estimate the parasite multiplication rate (PMR) and periodicity of the parasite cycle in the first week of patent parasitaemia using three different statistical methods:-
- standard two-stage method
  - global two-stage method
  - nonlinear mixed effects modelling
- b) To investigate the effect of different *P. falciparum* strains and sporozoite and blood induced infections on the parameters, PMR and periodicity.

### Aim 4 (Chapter 6)

- a) To estimate the population *in vivo* PD parameters of mefloquine using sequential PK-PD modelling of parasite count and mefloquine concentration data.
- b) To explore the demographic and disease related factors that influence the PD parameters.

### **3. POPULATION PHARMACOKINETICS OF MEFLOQUINE**

#### **3.1 Introduction**

In this chapter a population pharmacokinetic (PK) analysis is described which was performed on a large number of patient measurements to provide information on optimum dosage both when mefloquine is used alone and in combination with an artemisinin derivative. The effect of patient covariates on the PK of mefloquine was also investigated.

#### **3.2 Methods**

##### **3.2.1 Study population**

PK data were collected prospectively from patients receiving mefloquine as part of trials conducted between 1990 and 1995. Before 1994 mefloquine was given alone, and after 1994 it was given in combination with artesunate (12 mg/kg over 3 days).

The patients were from the Karen ethnic minority and lived in camps for displaced persons situated along the western border of Thailand in an area of low and seasonal malaria transmission (Luxemburger et al. 1996). Health care in these camps is provided by Médecins Sans Frontières (MSF).

Patients presenting to the clinics were enrolled into prospective studies of antimalarial treatment if they were symptomatic, with a microscopically confirmed infection with asexual stages of *P. falciparum* in the peripheral blood. Before 1994 the exclusion criteria for these studies were the following; weight less than 8kg, quinine administration within the previous 7 days, or mefloquine or sulfadoxine-pyrimethamine within the previous 14

days, pregnancy, signs of severity or concomitant disease requiring hospital admission (World Health Organisation, 1990). After 1994, on the basis of completed studies, these exclusion criteria were modified. Patients were excluded if any antimalarial medication had been given in the preceding 63 days, the parasitaemia at presentation was greater than 4%, their weight was less than 5kgs, or there were signs of severity or concomitant disease requiring hospital admission.

Informed consent was obtained from the patients, their parents or guardians. All studies were approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and by the Karen Refugee Committee, Mae Sod, Thailand.

### 3.2.2 Mefloquine dosing

All patients received one of the following two mefloquine (Lariam® 250 mg base tablets, Roche Pharmaceuticals, Basel, Switzerland) dosing regimens; 25 mg/kg (single dose) or 25 mg/kg (split dose – 15 mg/kg on day 0 followed by 10 mg/kg 24 hours later). Mefloquine was given to adults and older children to the nearest quarter tablet according to the calculated dose per kg of bodyweight. Younger children received mefloquine orally by syringe as crushed tablets, suspended in sugar water. For patients recruited prior to 1994 mefloquine was given alone and after 1994 mefloquine was combined with artesunate, 4 mg/kg/day for three days (50 mg tablets: Guilin No 1 factory, People's Republic of China).

### 3.2.3 Study procedures

On admission a questionnaire was completed recording details of symptoms and their duration, and the history of previous antimalarial medication. Since health structures in the camp are the only source of antimalarial drugs in this area, the history is generally a reliable guide to pre-treatment (Price et al. 1997). A full clinical examination was made and blood was taken for blood film, haematocrit, and white cell count. Malaria parasite

counts were determined on Giemsa-stained thick films as the number of parasites per 500 white blood cells. Patients were examined daily thereafter until they became aparasitaemic, and were then seen weekly for at least four weeks. At each clinic appointment a full physical examination was performed, the symptom questionnaire was completed, and, at the weekly visits, blood was taken for haematocrit and parasite count. Fever was defined as an axillary temperature greater than 37°C or an oral temperature greater than 37.5°C.

For those patients who received mefloquine monotherapy heparinised whole blood was collected either by fingerprick (100-200 µl of capillary blood) or venepuncture (500 µl of venous blood) on various days (ranging from day 0 to day 28) after the onset of treatment. For those patients who received combined therapy, where artesunate was administered on admission and mefloquine administered 2 days later, whole capillary blood samples (400 µl) were collected on days 0, 3, 5, 7, 9, 14 and 28. This corresponded to days 0, 1, 3, 5, 7, 12 and 26 following mefloquine administration. However for 27 of the subjects mefloquine and artesunate were both administered on admission and for these the blood samples were collected on days 0, 1, 3, 5, 7, 9, 14 and 28 following drug administration.

#### 3.2.4 Mefloquine assay

All blood samples were stored at -70°C until analysed. Whole blood mefloquine concentration measurements were performed by high performance liquid chromatography (HPLC) with ultraviolet detection as described previously (Edstein et al. 1991). Quantitation of mefloquine was performed by reversed-phase chromatography with isocratic elution. WR184,806 ( $\pm 2.8$  bis (trifluoromethyl) 4-[1-hydroxy-3-(*N*-tertbutylamino) propyl] quinoline phosphate) was used as an internal standard. Mefloquine was extracted by liquid-liquid extraction into dichloromethane layer, which was separated

from aqueous phase, evaporated to dryness and reconstituted in mobile phase before injection to HPLC (Edstein et al. 1991). Before 1994 whole blood was collected by either venepuncture (500  $\mu$ l) or by fingerprick (100-200  $\mu$ l). After 1994 all whole blood samples were collected by fingerprick (100-200  $\mu$ l). Whole blood samples were diluted two-fold with distilled water and then sonicated for 30 seconds before assay. The assay was linear over 0.05 to 4  $\mu$ g/ml. For the levels measured before 1994 (i.e. when mefloquine was given as a monotherapy) and split dosing, the inter-assay coefficients of variation (CV%) for the whole blood assays (200  $\mu$ l, n=19) were 6.1% and 3.6% at 100 and 1000 ng/ml respectively. For single dosing before 1994 the inter-assay coefficients of variation for the whole blood assays (200  $\mu$ l, n=15) were 6.3% and 5.7% at 100 and 1000 ng/ml respectively. For the levels recorded during 1994 and 1995 the inter-assay coefficients of variation for the capillary whole blood assays (n=44) were 8.3% and 5.7% at 100 and 1000 ng/ml respectively. The limit of detection of whole blood mefloquine was 50 ng/ml. At this study site capillary blood samples have been shown to give equivalent results to larger venous blood samples (ter Kuile et al. 1994).

### 3.2.5 Pharmacokinetic model

For the development of the population PK model, patients with detectable whole blood concentrations of mefloquine on the day of admission to study were excluded.

The data could only support, for orally administered mefloquine, a one-compartment model (Gibaldi and Perrier, 1982) with first-order absorption and first-order elimination. The equation for plasma concentration as a function of time after oral administration is bi-exponential.

$$C = \left[ \frac{k_a \cdot \text{dose} \cdot F}{V \cdot k_a - CL} \right] * \left[ e^{-\left(\frac{CL}{V}\right)t} - e^{-k_a \cdot t} \right]$$

where	C	- mefloquine concentration (ng/ml)
	$k_a$	- first order absorption rate constant (/day)
	dose	- dosage of drug given ( $\mu\text{g/kg}$ )
	F	- fraction of drug absorbed
	V	- total apparent volume of distribution (l/kg)
	CL	- clearance (l/kg/day)
	t	- time (days)

The data in this part of the analysis came originally from standard PK studies where serial samples were taken at the same times for each subject and a standard two-stage PK analysis performed (ter Kuile et al. 1993b; Price et al. 1999a). The first mefloquine level measured was taken on average 24 hours (range 20-31 hours) after drug intake so there was minimal information regarding the absorption phase. As absolute bioavailability cannot be determined the fundamental PK parameters used to characterise the above one-compartment model were apparent clearance (CL/F) and apparent volume of distribution (V/F). The absorption rate constant was set to a constant value of 7.0 /day derived from a PK study (Nosten et al. 1991a) of malaria patients from the same population as the present study.

### 3.2.6 Statistical model

The residual intra-individual error was modelled using an additive error model. That is the departure of an individual concentration from the predicted population value is independent of the actual concentration.

$$C_{ij} = C_{pij} + \varepsilon_{ij}$$



$C_{ij}$  is the  $j^{\text{th}}$  observed concentration for the  $i^{\text{th}}$  individual and  $C_{pij}$  is the  $j^{\text{th}}$  predicted concentration for the  $i^{\text{th}}$  individual.  $\epsilon_{ij}$  is the residual intra-subject error term and is assumed to be randomly Normally distributed with zero mean and variance  $\sigma^2$ .

Inter-individual variability in apparent clearance (CL/F) and volume of distribution (V/F) were modelled with log normal error models. A log-normal error model differs from an additive error model because the model assumes departure of the individual PK parameter (either CL/F<sub>i</sub> or V/F<sub>i</sub>) from the population mean PK parameter (either CL/F or V/F) is dependent on the value of the PK parameter, not constant across all individuals. Log normal error models also ensure the individual PK parameters, (CL/F)<sub>i</sub> and (V/F)<sub>i</sub>, must be greater than zero.

$$(CL / F)_i = (CL / F) \cdot \exp(\eta_i^{CL/F}) \quad (3.1)$$

$$(V / F)_i = (V / F) \cdot \exp(\eta_i^{V/F}) \quad (3.2)$$

CL/F<sub>i</sub> and V/F<sub>i</sub> are the PK parameters for the individual and CL/F and V/F are the population means. The  $\eta_i^{CL/F}$  and  $\eta_i^{V/F}$  are random effects with zero mean and variances  $\sigma_{CL/F}^2$  and  $\sigma_{V/F}^2$ , respectively, which represent the inter-individual variability for each of the parameters. The inter-individual variability of CL/F and V/F were expressed as asymmetric 90% prediction intervals: for example for apparent clearance,  $\exp [\log_e(CL/F) \pm 1.645 \cdot \sigma_{CL/F}]$ .

### 3.2.7 Data analysis

The NLME procedure (Lindstrom and Bates, 1990) of the SPLUS data programme (SPLUS 4.5 for Windows, Mathsoft, Inc.) was used to calculate estimates of the population

PK parameters (CL/F, V/F) and their respective inter-individual variances ( $\eta^{CL/F}$ ,  $\eta^{V/F}$ ). The programme also provides estimates of the residual random intra-individual error ( $\epsilon$ ) and the variances of the inter-individual error terms ( $\sigma_{CL/F}^2$ ,  $\sigma_{V/F}^2$ ). Convergence was achieved when, between iterations, the objective value did not differ by more than some pre-specified difference (eg 0.0001) and the programme returned the final estimates of the population PK parameters. The objective function (minus twice the log-likelihood of the data) was used to determine the model that best fitted the data. A significant drop in the objective function (using the Chi-squared distribution with degrees of freedom equal to number of parameters which are set equal to a fixed value in the restricted model) from the general model to the restricted model was used to determine the final PK model. The goodness of fit of each model was also determined by the precision of the parameter estimates and examination of the scatter plot of residuals versus predicted mefloquine levels. The individual PK parameters were calculated using the posterior estimates.

Covariates were used to attempt to explain some of the inter-individual variability. Multiple linear regression was used to investigate the relationship between the fixed effects (i.e. demographic, laboratory and clinical data) and the random effects (i.e. the PK parameters). The PK parameters were the dependent variables and the fixed effects were entered into the regression model as the independent variables using stepwise methods. The statistical computations were performed using the statistical programme SPSS Version 7.5 for Windows (SPSS Inc., Chicago, IL.).

Covariates that were found to be significant in the above multiple linear regression analyses were introduced into the one compartment model by being incorporated into equations (3.1) and (3.2) as the following:-

$$(CL / F)_i = [(CL / F) + \theta_1.(HF)].\exp(\eta_i^{CL / F})$$

$$(V / F)_i = [(V / F) + \theta_2.(TI)].\exp(\eta_i^{V / F})$$

HF – history of fever = 1 if history of fever  $\geq 2$  days;

= 0 if history of fever  $< 2$  days;

TI – type of infection = 1 if mixed infection;

= 0 if pure *P. falciparum* infection.

$\theta_1$  represents the change in CL/F due to a prolonged history of fever

and  $\theta_2$  represents the change in V/F due to a mixed infection.

Covariates which were continuous variables were centred around their mean values so that the population estimates would represent those of an average patient.

For example:-  $(CL / F)_i = [(CL / F) + \theta_3.(HCT - \text{mean}(HCT))].\exp(\eta_i^{CL / F})$

where HCT = haematocrit

$\theta_3$  represents the change in CL/F due to haematocrit from the population mean value of CL/F for patients with a haematocrit equal to the mean value.

A statistically significant improvement in the objective function, improvement in the precision of the parameter estimate (SE), and reduction in inter-patient and residual variability were used to determine the importance of the covariates as significant predictors.

### 3.2.8 Efficacy modelling

The variables chosen prospectively to represent therapeutic response were clearance of parasites and fever within 24 and 48 hours, and recrudescence of the infection (treatment failure) during 28 days of follow up. Multiple logistic regression was performed to model

these binary outcomes. The independent variables included in the analysis were admission parasitaemia (log transformed), admission temperature, age, type of dosage (split versus single), and individual estimates of  $AUC_{0-\infty}$  and V/F.

### **3.3 Results**

Overall 1020 whole blood mefloquine concentrations (excluding 0 hr samples) taken from 257 patients with acute falciparum malaria between 1990 and 1995 were available for analysis. Of these, 513 mefloquine concentrations were from 159 subjects who were given a split dose: 15 mg/kg initially followed by 10 mg/kg 24 hours later. The remaining 507 mefloquine concentrations were from subjects to whom a single dose of 25 mg/kg was administered. The demographic characteristics of the patients are given in Table 3.1. The majority of patients (83%) were followed weekly for at least four weeks.

#### **3.3.1 Population pharmacokinetic model**

The model that gave the best fit to the data was a one compartment model with first order absorption and with CL/F and V/F fitted as random effects. That is, equations (3.1) and (3.2) were fitted by the NLME procedure. To avoid convergence problems with the optimisation algorithm used in NLME due to the sparsity of the mefloquine concentrations in the data the random effects of CL/F and V/F were assumed to be independent.

Appendices I and II give the SPLUS programmes used for fitting the one compartment model to split and single dosing respectively.

**Table 3.1. Admission characteristics for all patients.**

	Mefloquine alone		Mefloquine + Artesunate	
	Split dose (Group A)	Single dose (Group B)	Split dose (Group C)	Single dose (Group D)
No. of Subjects Enrolled	128	24	31	74
Year of Study	1990 – N=63	1990 – N=0	1994 – N=27	1994 – N=28
	1991 – N=65	1991 – N=24	1995 – N=4	1995 – N=46
Males N (%)	76 (59)	13 (54)	19 (61)	41 (55)
Age (years) <sup>a</sup>	14.8 (12.7-16.8) [1.2-54]	17.9 (12.8-23) [0.8-51]	9.1 (8.1-10) [5-14]	9.3 (8.6-9.9) [4-15]
< 5 N (%)	15 (12)	3 (13)	0	3 (4)
5-14 N (%)	67 (52)	7 (29)	31 (100)	69 (93)
>14 N (%)	46 (36)	14 (58)	0	2 (3)
Weight (kg) <sup>a</sup>	31.0 (28.3-33.7) [8-61]	35.9 (29.2-42.7) [9-59]	24.0 (21.0-27.1) [14-42]	25.6 (23.8-27.5) [13-43]
Primary Infection N (%)	90 (70)	23 (96)	31 (100)	74 (100)
Fever on admission N (%)	91 (71)	16 (67)	18 (58)	48 (65)
Prolonged history of fever N (%)	80 (74)*	18 (75)	16 (52)	35 (47)
Pure <i>P.falciparum</i> infection N (%)	110 (91)*	20 (83)	20 (65)	47 (64)
Haematocrit (%) <sup>a,*</sup>	34.8 (33.5-36.2) [15-55]	34.2 (31.8-36.5) [21-44]	35.7 (34.1-37.2) [22.8-41.4]	35.7 (34.6-36.8) [22.8-44.9]
Haematocrit < 30% N(%)	14 (18)	1 (5)	2 (7)	6 (9)
Parasite count per µl blood <sup>b</sup>	3486 (2516-4832)	3409 (1614-7200)	7204 (3719-13,954)	7730 (4727-12641)
	[64-146,080]	[50-114,660]	[55-105,152]	[25-176,832]
>10,000 /µl N (%)	39 (31)	8 (33)	14 (45)	32 (43)

a – mean (95% CI) [range], b – geometric mean (95% CI) [range],

\* - missing data for some patients (for haematocrit: Group A n=79; Group B n=19; Group C n=29; Group D n=67)

The one compartment model was fitted separately to the following four datasets,

monotherapy & split dosing (group A),

monotherapy & single dosing (group B),

combined therapy & split dosing (group C),

combined therapy & single dosing (group D),

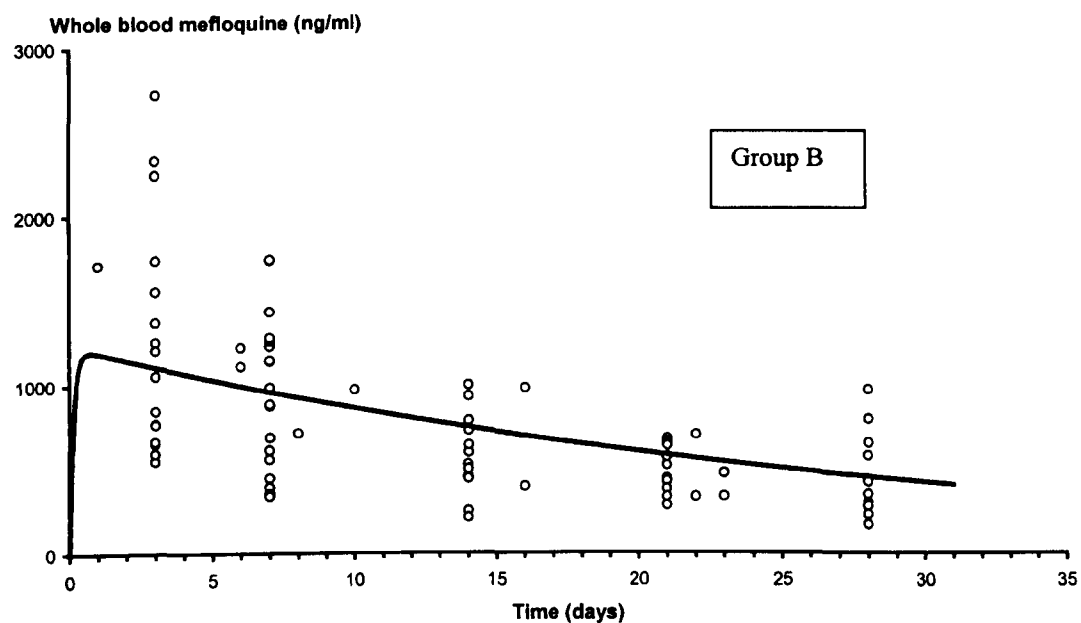
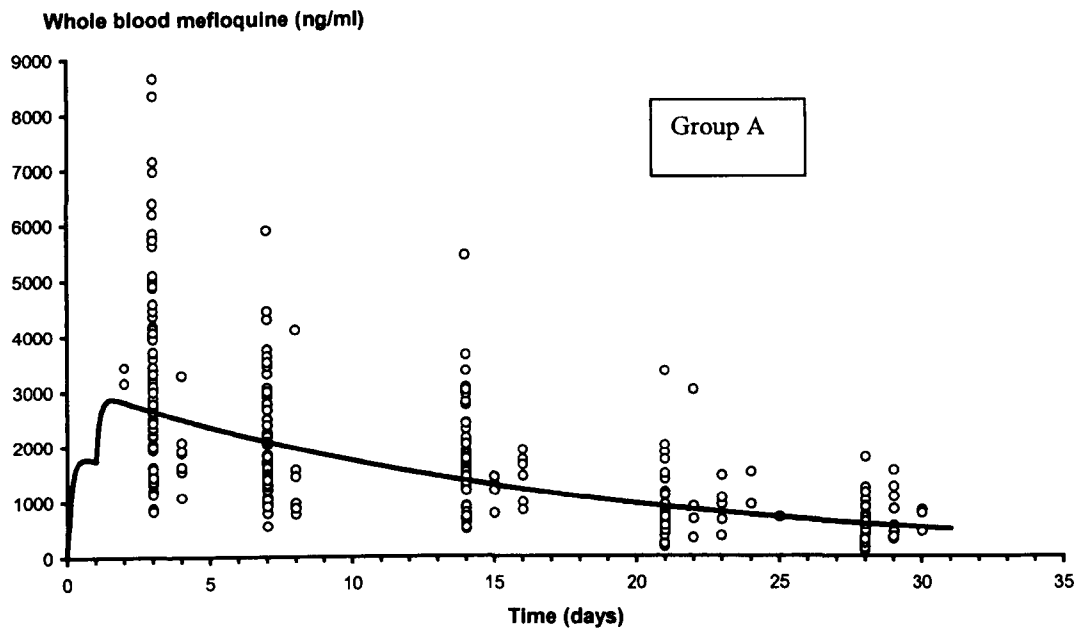
since they exhibited very different PK profiles and had variability in the magnitude of their residual error.

All mefloquine levels were assayed using capillary blood for datasets C and D. However, for datasets A and B, 53% (186/350) and 65% (53/82) of the mefloquine levels respectively were assayed from venous blood with the remainder analysed from capillary blood. There were no significant differences between mefloquine levels from venous and capillary blood for each of the sampling times (day 3, 7, 14, 21 and 28) or for the same patient on any one day (ter Kuile et al. 1994). However, the magnitude of the residual variability was greater for concentrations measured from capillary blood as opposed to the venous blood. To adjust for this a covariate was attached to the residual error. This had little effect on the estimates of CL/F and V/F, but did improve the objective function significantly.

Figures 3.1a, 3.1b, 3.1c and 3.1d show scatter plots of the observed values and resulting population PK profiles versus time for the four different datasets. As shown in Figure 3.2 the concentration-time profiles for the four datasets were very different. The patient specific profiles were characterised adequately (Figure 3.3) and the residual plots indicated no bias in estimation (Figures 3.4a, 3.4b, 3.4c, 3.4d). The mean population PK parameters of all four base models are presented in Table 3.2. Inter-individual variability, defined as 90% prediction intervals of the population estimates, varied greatly for both CL/F and V/F.

**Table 3.2. Population pharmacokinetic parameters for the base model.**

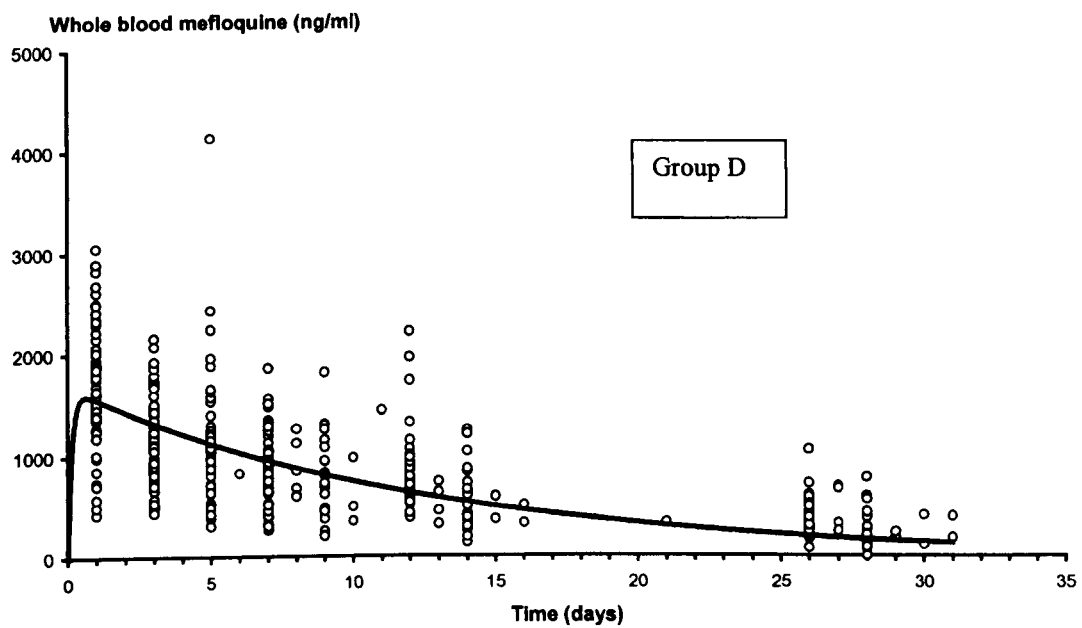
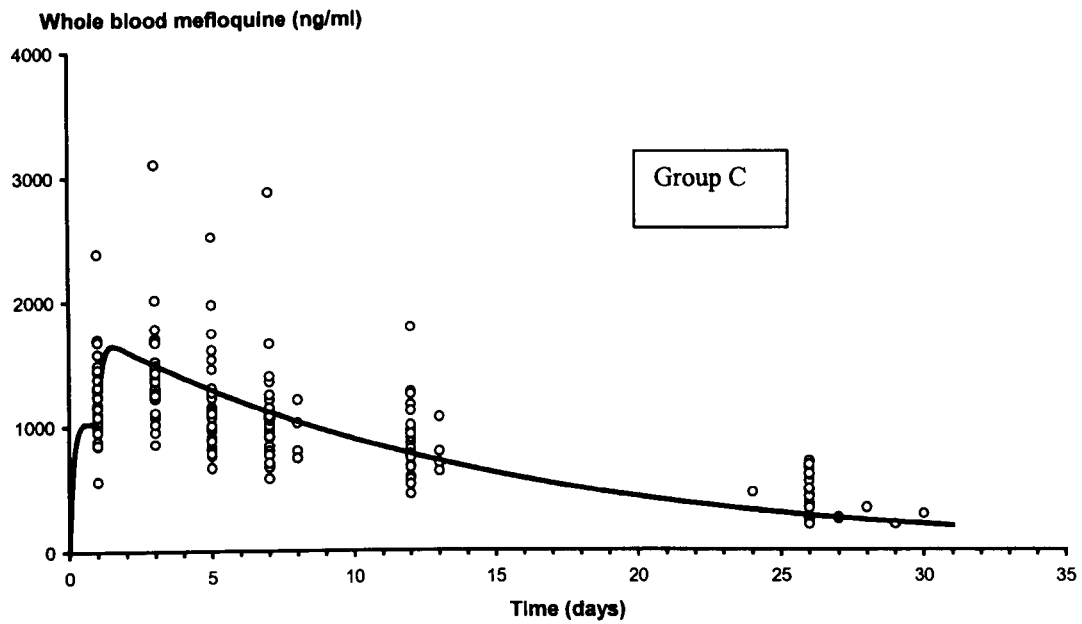
	Mefloquine monotherapy		Combined therapy	
	Estimate (SE)	90% Prediction Intervals.	Estimate (SE)	90% Prediction Intervals.
Split dosing	Group A		Group C	
CL/F (l/kg/day)	0.490 (0.023)	0.254, 0.946	1.027 (0.083)	0.600, 1.759
V/F (l/kg)	8.14 (0.43)	3.66, 14.43	13.99 (0.70)	9.81, 19.95
k <sub>e</sub> (elimination rate)	0.060 /day		0.073 /day	
t <sub>1/2</sub> (elimination half life)	11.6 days		9.5 days	
AUC <sub>0-∞</sub> (area under curve)	51020 ng/ml*day		24343 ng/ml*day	
σ <sub>e</sub> (ng/ml)	697.4 (for capillary) 219.5 (for venous)		262.5 (for capillary)	
Single dosing	Group B		Group D	
CL/F (l/kg/day)	0.733 (0.051)	0.572, 0.939	1.232 (0.082)	0.490, 3.101
V/F (l/kg)	20.37 (2.53)	8.09, 51.32	14.95 (0.66)	8.64, 25.86
k <sub>e</sub> (elimination rate)	0.036 /day		0.082 /day	
t <sub>1/2</sub> (elimination half life)	19.3 days		8.5 days	
AUC <sub>0-∞</sub> (area under curve)	34106 ng/ml*day		20292 ng/ml*day	
σ <sub>e</sub> (ng/ml)	180.4 (for capillary) 167.6 (for venous)		294.7 (for capillary)	



**Figures 3.1a & 3.1b.** Whole blood mefloquine concentrations in acute falciparum malaria. The *solid line* shows the fit of the data and represents the predicted concentration-time profile for the population mean.

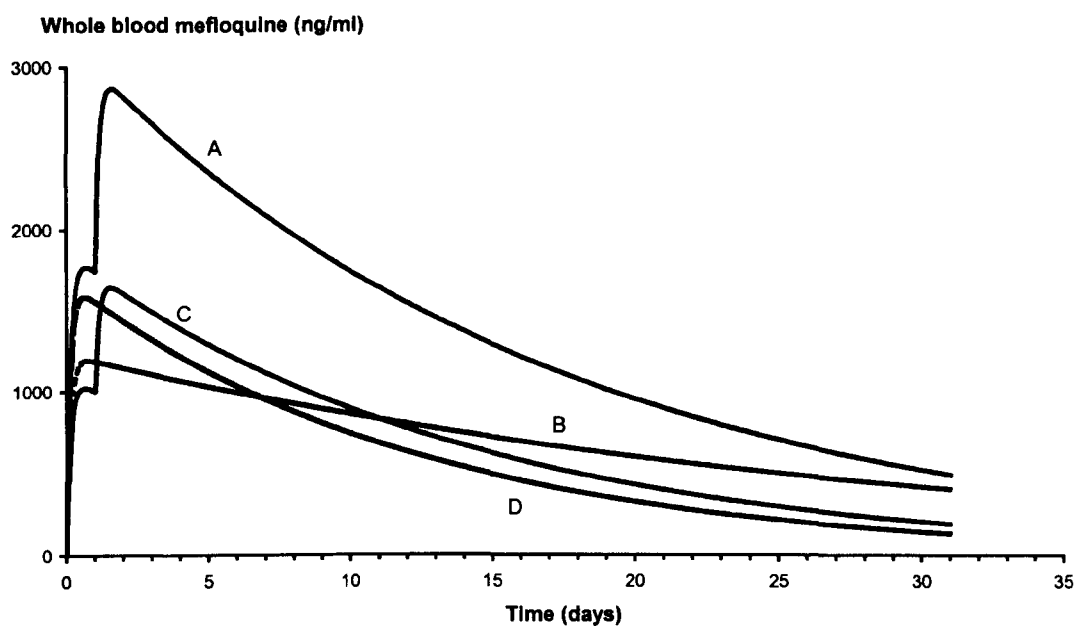
**A:** mefloquine monotherapy: split dosing **B:** mefloquine monotherapy: single dosing





**Figures 3.1c & 3.1d.** Whole blood mefloquine concentrations in acute falciparum malaria. The *solid line* shows the fit of the data and represents the predicted concentration-time profile for the population mean.

**C:** mefloquine & artesunate: split dosing **D:** mefloquine & artesunate: single dosing



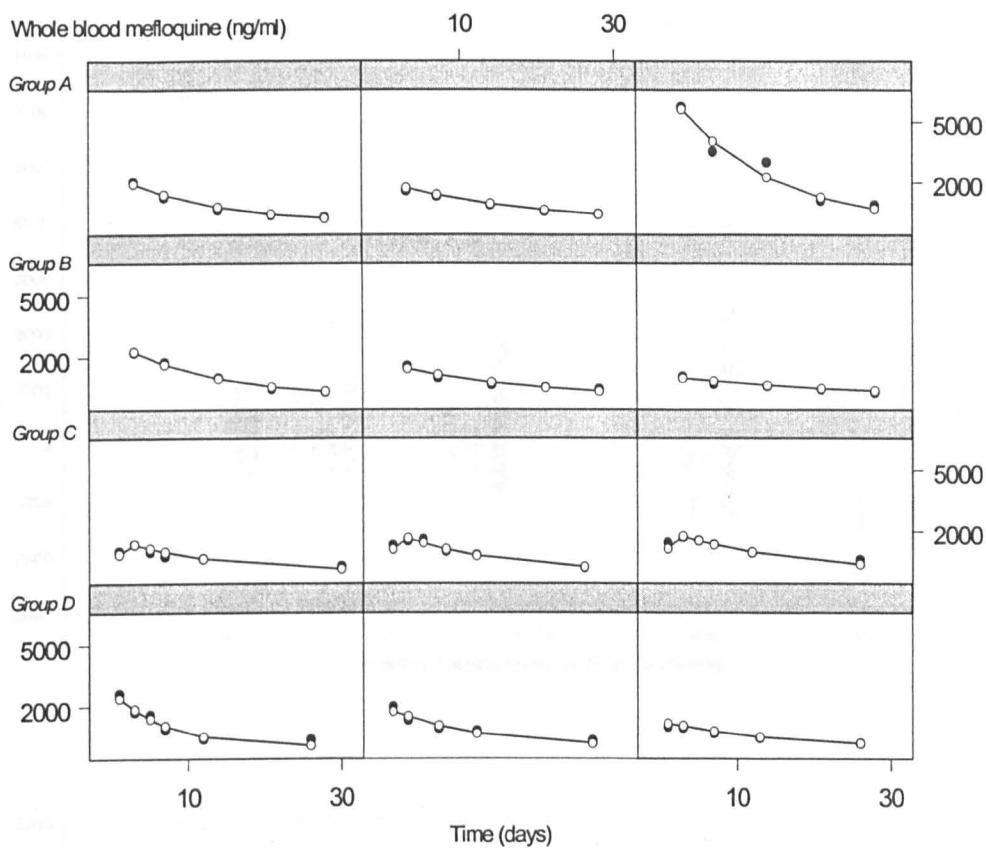
**Figure 3.2.** Predicted population pharmacokinetic profiles for datasets,

**A:** mefloquine monotherapy: split dosing;

**B:** mefloquine monotherapy: single dose

**C:** mefloquine & artesunate: split dosing;

**D:** mefloquine & artesunate: single dosing



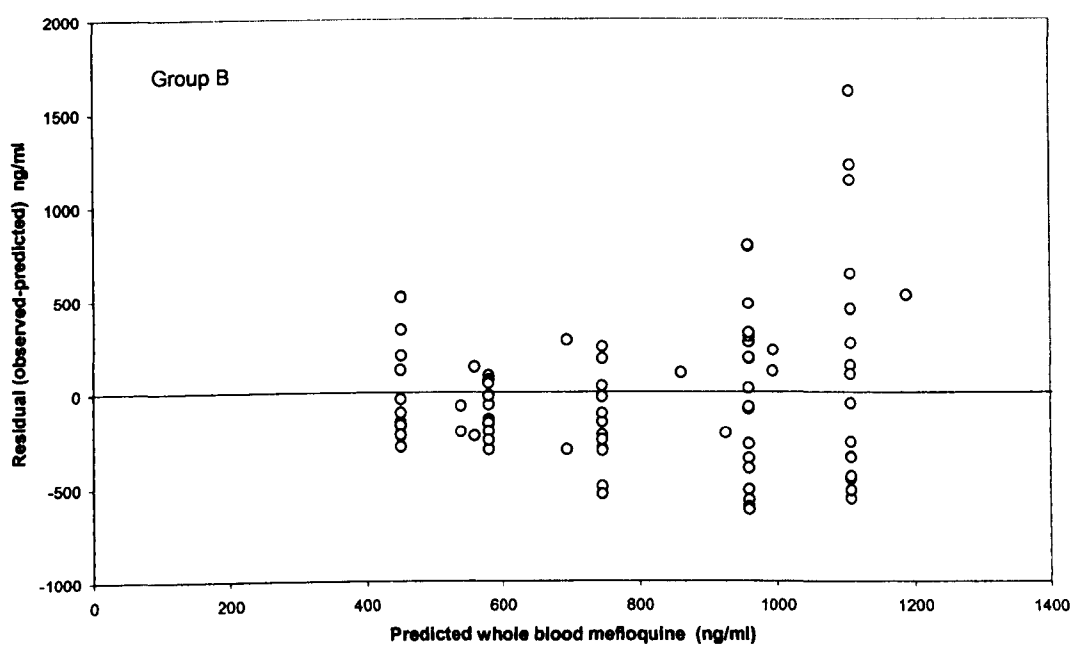
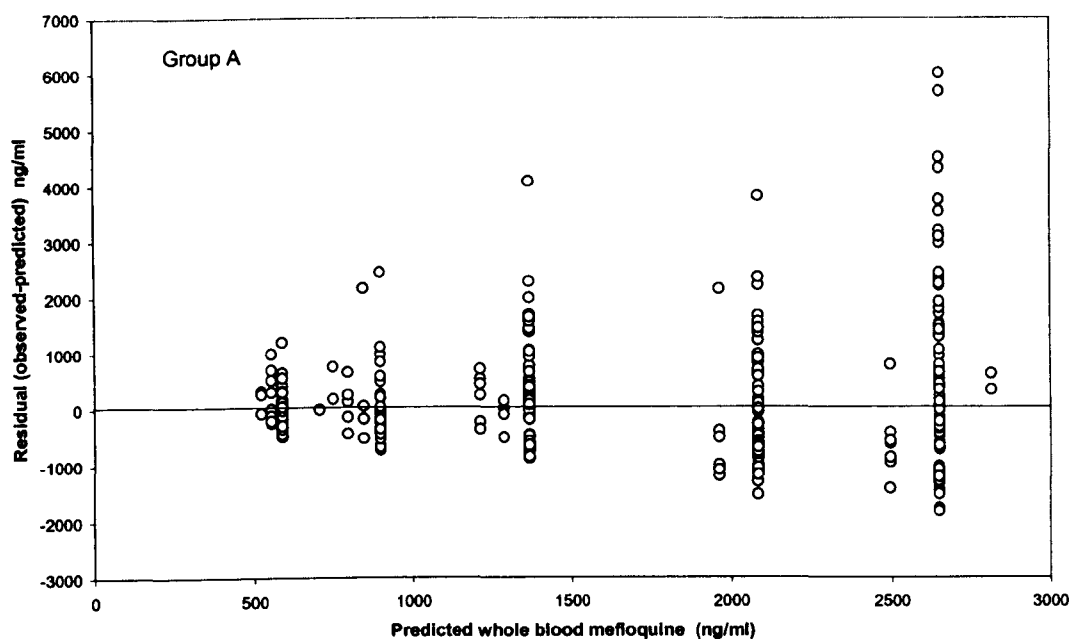
**Figure 3.3.** Patient specific profiles.

[Row 1 – group A; Row 2 – group B; Row 3 – group C; Row 4 – group D]

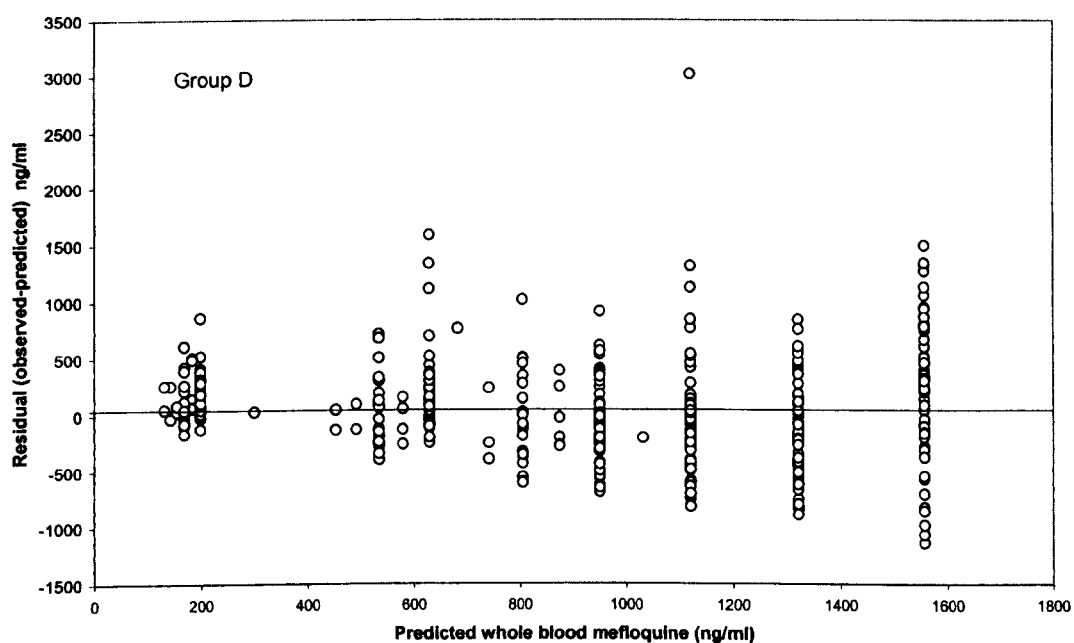
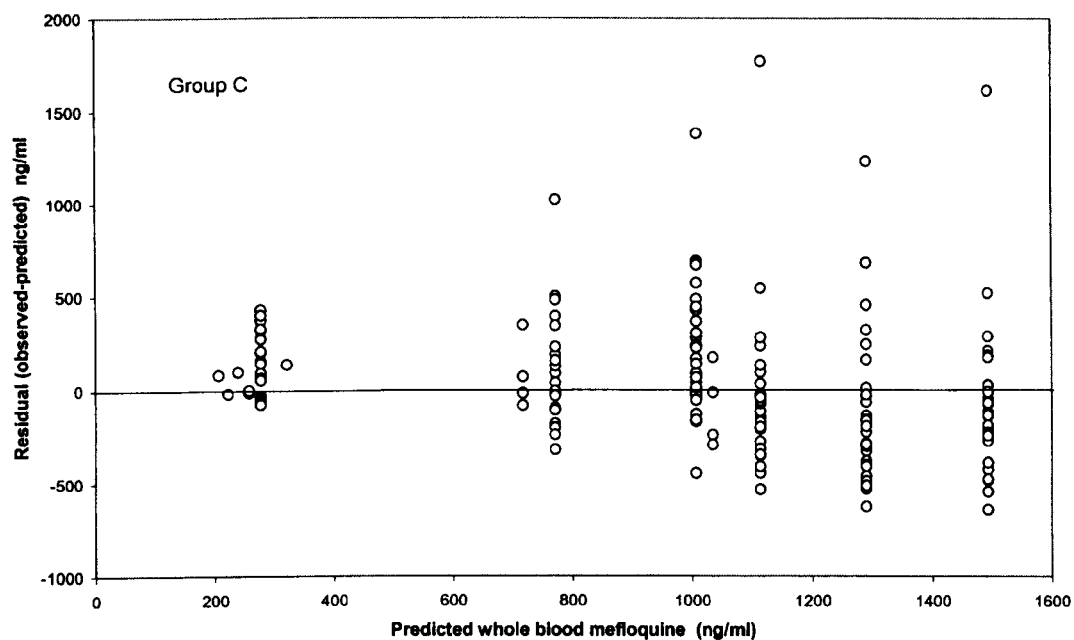
*x-axis* – time (days);

*y-axis* – whole blood mefloquine (ng/ml).

Closed circles – observed data; Solid line – predicted data.



**Figures 3.4a & 3.4b.** Assessment of goodness of fit: Plot of absolute residuals versus predicted whole blood mefloquine (ng/ml). For **A**: mefloquine monotherapy: split dosing & **B**: mefloquine monotherapy: single dosing



**Figures 3.4c & 3.4d.** Assessment of goodness of fit: Plot of absolute residuals versus predicted whole blood mefloquine (ng/ml). For **C**: mefloquine & artesunate: split dosing & **D**: mefloquine & artesunate: single dosing

### 3.3.2 Split versus single dosing

The area under the concentration time curve ( $AUC_{0-\infty}$ ) was calculated from the population estimate of CL/F for groups A, B, C and D. Splitting the dose of mefloquine increased the  $AUC_{0-\infty}$  by 50% (95% CI: 36 to 65%) compared to a single dose for monotherapy and by 20% (95% CI: 3 to 40%) for the combined therapy.

Estimated V/F was significantly smaller for subjects receiving split dosing compared to those receiving single dosing (estimate (95% CI): 8.14 (7.49, 8.86) vs 20.37 (16.26, 25.51) l/kg,  $p < 0.001$  respectively) when mefloquine was given as a monotherapy. However, when mefloquine was given as combined therapy no significant differences in V/F were observed between those subjects receiving split dosing and those receiving a single dose (estimate (95% CI): 13.99 (9.16, 21.36) vs 14.95 (7.78, 28.71) l/kg,  $p = 0.305$  respectively).

To check the above findings all four datasets were combined and formal hypothesis testing was performed to investigate the effects of the covariates: monotherapy versus combined therapy and split versus single dose. The results obtained confirmed the above findings.

### 3.3.3 Relationship between pharmacokinetic parameters and covariates

The covariates deemed important prospectively were age, weight, sex, temperature, haematocrit, admission parasitaemia, type of infection (pure *P. falciparum*, or mixed *P. falciparum* and *P. vivax*), vomiting or diarrhoea on admission; prolonged history of fever (fever for two or more days before admission); and history of vomiting and fever. However, only three subjects had diarrhoea on admission, so this variable was excluded from all analyses. For group D the covariate termed 'day of mefloquine administration' was included in the analysis to see if there was any change in the PK of mefloquine when a

single dose of mefloquine was given on day 0 compared to day 2. Of the 74 subjects 27 (36.5%) were given mefloquine on day 0.

No association between the body-weight normalised PK parameters and age or weight was observed for any of the four groups. Univariate associations between the PK parameters and the following independent variables; temperature, haematocrit, parasitaemia, vomiting and type of infection on admission, and a long history of fever were observed. For group D there was a significant association between apparent clearance and day of mefloquine administration.

Thus stepwise multiple linear regression was performed to investigate the relationship between CL/F and V/F and the various covariates.

Dependent variables-	CL/F, V/F
Independent variables-	age, admission temperature, admission haematocrit, admission parasitaemia, long history of fever, pure <i>P. falciparum</i> infection on admission, vomiting on admission, day of mefloquine administration (only for group D).

Table 3.3 summarises the covariates that were independent predictors of both CL/F and V/F for each of the four groups. Figures 3.5a, 3.5b, 3.5c, 3.5d, 3.5e, 3.5f and 3.5g are either scatter plots or error bar plots where appropriate of the posterior PK parameters against the independent predictors summarised in Table 3.3. For group A none of the above variables were found independently to be significant predictors of CL/F. For group B no independent associations between both CL/F and V/F and any of the variables were found.

**Table 3.3. Final regression models for CL/F and V/F from multiple linear stepwise regression.**

Group	Regression model	R <sup>2</sup>
A	V/F = 48.75 – 1.060*temperature	6.5%
C	CL/F = 0.068 + 0.028*haematocrit	24%
	V/F = 3.47 + 0.30*haematocrit	31%
D	CL/F = 1.376 + 0.217*(long history of fever) – 0.125*(day of administration)	12.5%
	V/F = 13.36 + 2.28*(long history of fever) + 3.21*(infection type)	13.5%

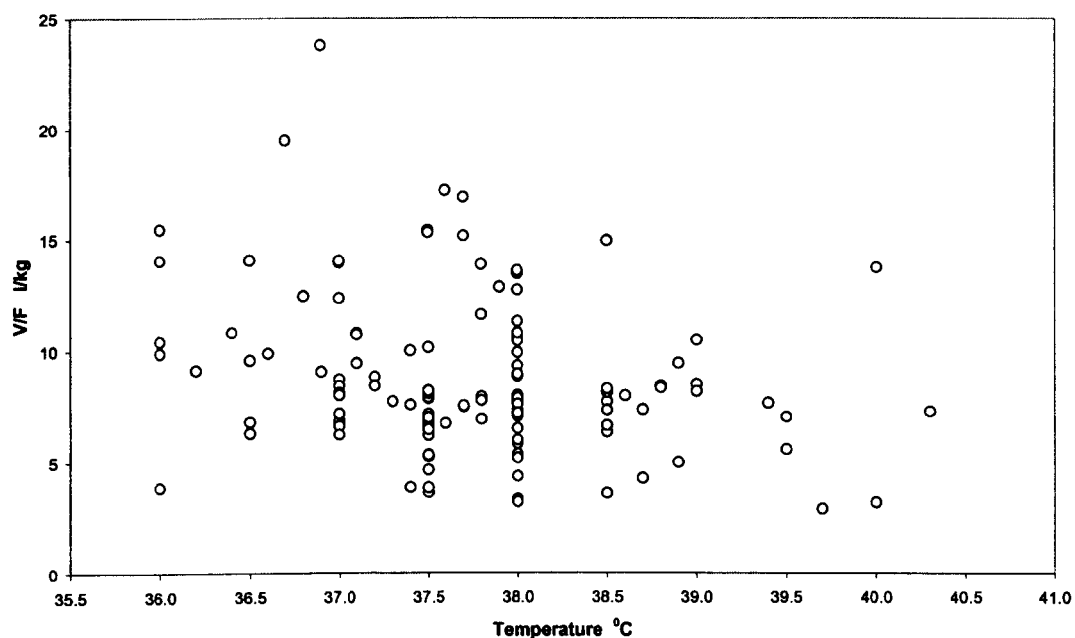
Footnote: R<sup>2</sup> – percentage of the total variation in the dependent variable (i.e. V/F or CL/F) explained by the regression model

long history of fever      = 1 if history of fever ≥ 2 days  
                                      = 0 if history of fever < 2 days

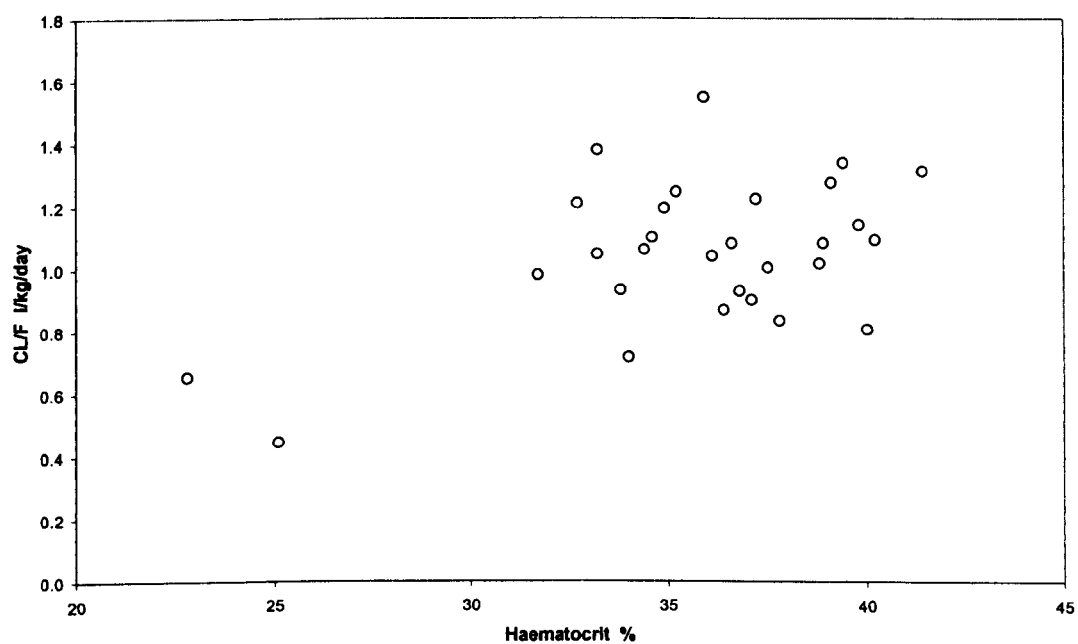
day of administration      = 0 if mefloquine given on day of admission  
                                      = 1 if mefloquine given 2 days after admission

infection type                = 1 if mixed infection  
                                      = 0 if pure *P. falciparum* malaria

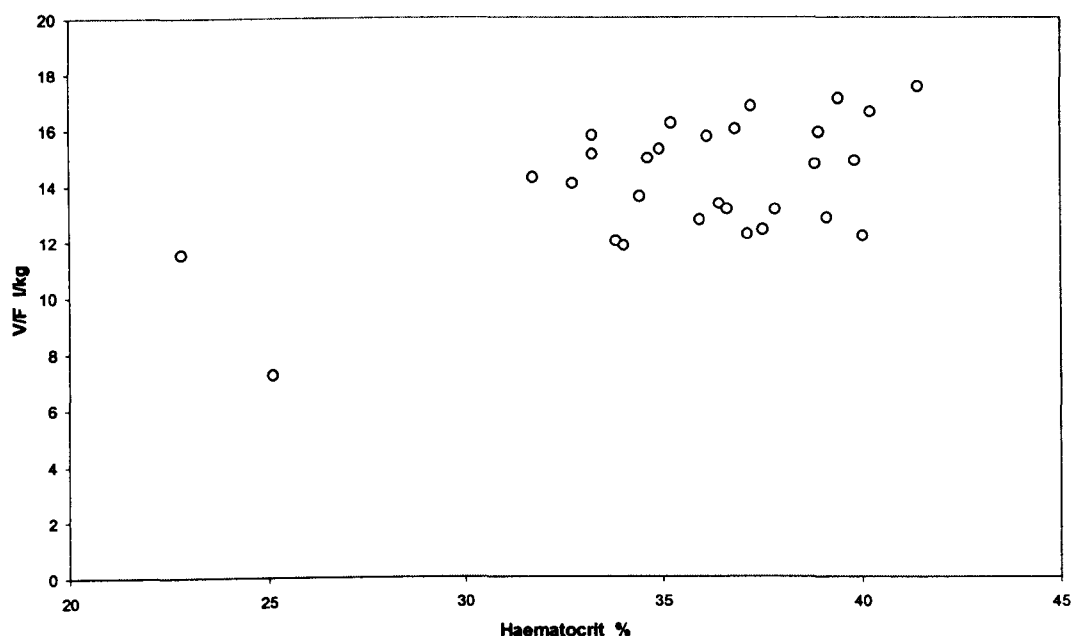




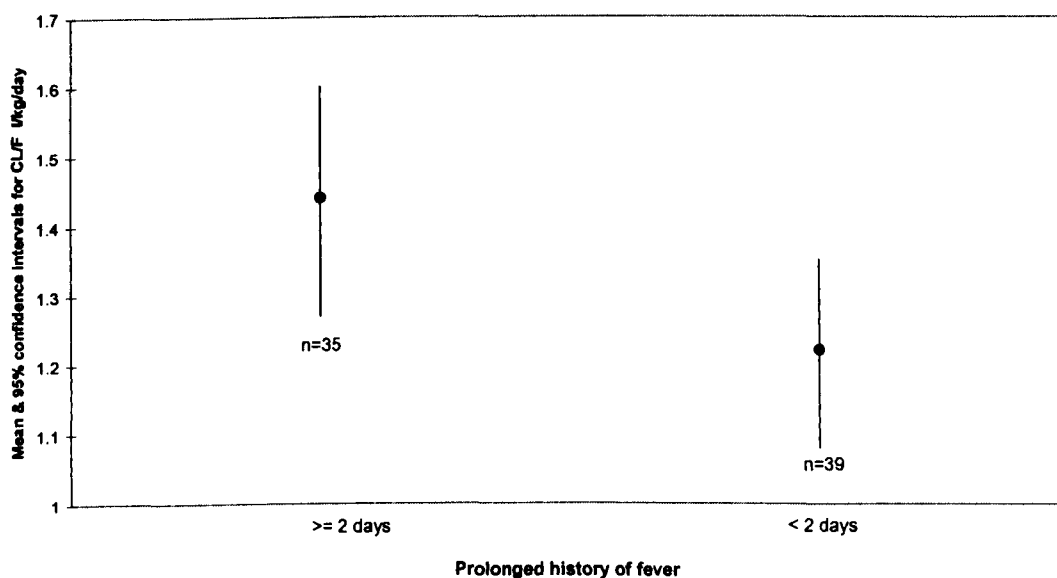
**Figure 3.5a.** Relationship between posterior estimates of V/F (l/kg) and admission temperature in patients from group A (mefloquine monotherapy: split dosing).



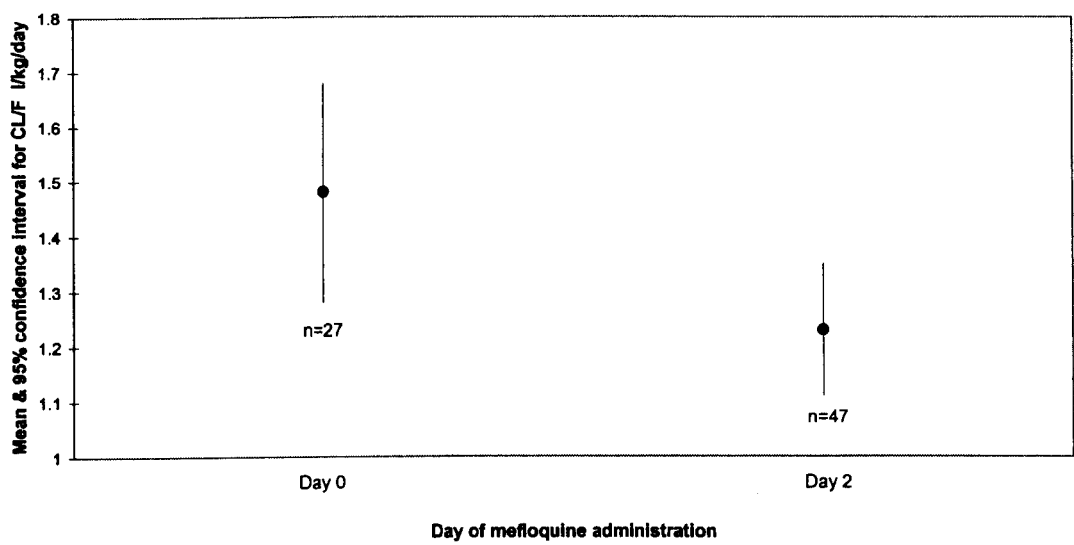
**Figure 3.5b.** Relationship between posterior estimates of CL/F (l/kg/day) and admission haematocrit in patients from group C (mefloquine & artesunate: split dosing).



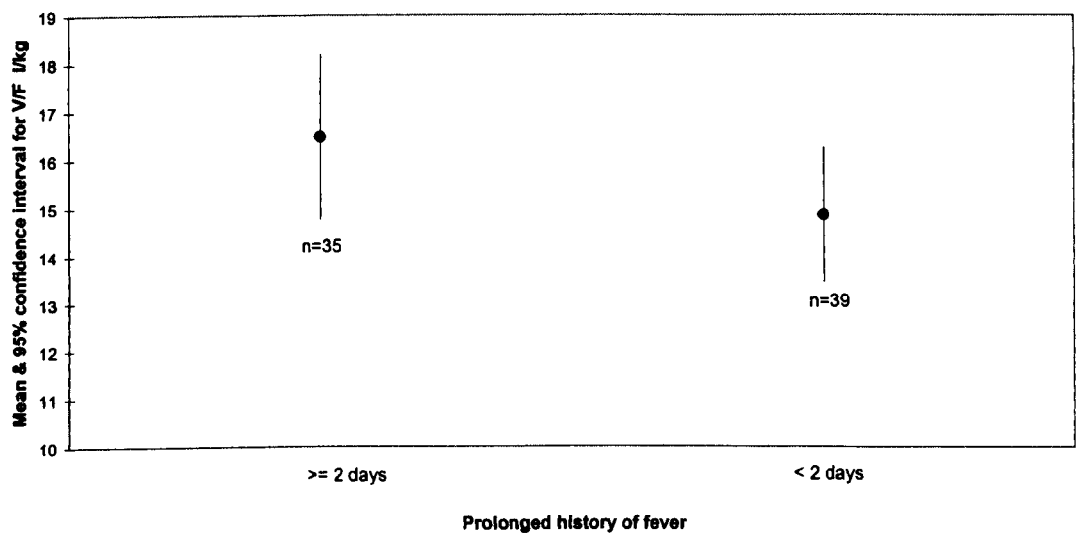
**Figure 3.5c.** Relationship between posterior estimates of V/F (l/kg) and admission haematocrit in patients from group C (mefloquine & artesunate: split dosing).



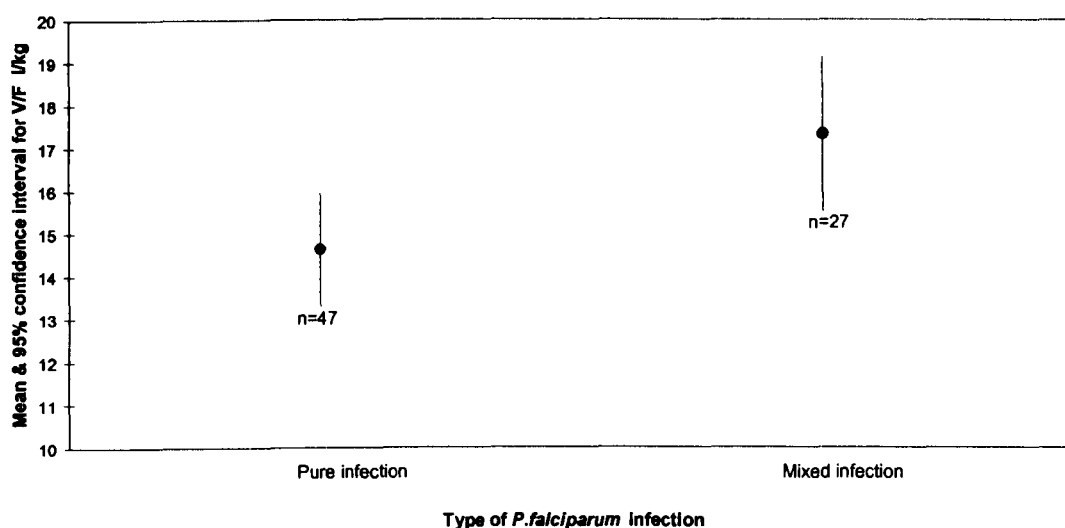
**Figure 3.5d.** Relationship between posterior estimates of CL/F (l/kg/day) and prolonged history of fever in patients from group D (mefloquine & artesunate: single dosing).



**Figure 3.5e.** Relationship between posterior estimates of CL/F (l/kg/day) and day of mefloquine administration in patients from group **D** (mefloquine & artesunate: single dosing).



**Figure 3.5f.** Relationship between posterior estimates of V/F (l/kg) and prolonged history of fever in patients from group **D** (mefloquine & artesunate: single dosing).



**Figure 3.5g.** Relationship between posterior estimates of V/F (l/kg) and type of *P. falciparum* infection in patients from group D (mefloquine & artesunate: single dosing).

### 3.3.4 Final model estimates

From the multiple linear regression analysis the covariates found to be independently associated significantly with the individual estimates of CL/F and V/F were now added univariately to the PK model. Table 3.4 summarises the effects of each covariate on the PK model for groups A, C and D.

**Table 3.4. Summary of results with incorporation of covariates into the base model.**

	Parameter estimate	95% CI	Change in objective function	p-value
<b>Group A</b>				
Effect of temperature on V/F	-1.392	-2.23, -0.55	8.38	<0.01
<b>Group C</b>				
Effect of haematocrit on CL/F,	0.012	-0.001, 0.025	2.74	>0.05
Effect of haematocrit on V/F.	0.085	-0.017, 0.188	2.49	>0.05
<b>Group D</b>				
Effect of prolonged history of fever on CL/F,	0.310	-0.019, 0.639	3.17	>0.05
Effect of day of mefloquine administration on CL/F,	-0.174	-0.346, -0.002	4.09	0.01<p<0.05
Effect of prolonged history of fever on V/F,	2.06	-0.55, 4.67	2.60	>0.05
Effect of mixed infection on V/F.	3.82	1.00, 6.64	7.38	<0.01

It can be seen that the covariates had minimal effect on the fit of the PK model. For group A, the minimum, mean and maximum admission temperatures observed were 36, 37.7 and 40.3°C respectively. At these temperatures the respective population estimates of V/F would be 10.6, 8.21 and 4.59 l/kg. Thus in the most extreme scenario (i.e. a patient with a subnormal temperature of 36 °C) there would be an approximate 29% increase in V/F from the population mean. For group D the inclusion of the covariate ‘day of mefloquine administration’ improved the objective function of the PK model marginally. Administering mefloquine on day 2 (i.e. during recovery) decreased the CL/F by 0.174 l/kg/day (95% CI: 0.002, 0.346). For group D a mixed infection was shown to increase the V/F by 3.82 l/kg (95% CI: 1.00, 6.64). Table 3.5 presents the final population parameter estimates and the inter-individual and residual variability for groups A and D where the addition of covariates improved the objective function significantly.

**Table 3.5. Population pharmacokinetic parameters for the final models in the two groups where adjusting for covariates gave improved fits.**

	Mefloquine monotherapy & split dosing.		Combined therapy & single dosing.	
	Estimate (SE)	90% Prediction Intervals.	Estimate (SE)	90% Prediction Intervals.
	<b>Group A<sup>m</sup></b>		<b>Group D<sup>n</sup></b>	
CL/F (l/kg/day)	0.487 (0.023)	0.253, 0.936	1.278 (0.089)	0.602, 2.714
Factor for day of drug administration during convalescence on CL/F			-0.18 (0.09)	
V/F(l/kg)	8.21 (0.43)	3.83, 17.61	13.71 (0.72)	8.25, 22.79
Change in V/F (l/kg) due to drop in 1°C of admission temperature	1.39 (0.43)			
Factor for mixed infection on V/F			3.88 (1.43)	
k <sub>e</sub> (elimination rate)	0.060 /day		0.082 /day	
t <sub>1/2</sub> (elimination half life)	11.6 days		8.5 days	
AUC (area under curve)	51335 ng/ml*day		19562 ng/ml*day	
σ <sub>e</sub> (ng/ml)	696.0 (for capillary) 219.3 (for venous)		294.8 (for capillary)	

m - Incorporates the effects of admission temperature on V/F.

n - Incorporates the effects of both day of drug administration on CL/F and type of infection on V/F.

### 3.3.5 Weight adjusted dose

For the studies conducted post 1994 (Groups C & D) the actual dose of mefloquine given was recorded. Using the actual dose (mg) in the model as opposed to the weight adjusted dose (i.e. a single dose of 25 mg/kg or a split dose of 15mg/kg on day 0 followed by 10 mg/kg 24 hours later), the relationship between the PK parameters (CL/F and V/F) and body weight was investigated.

#### *Mefloquine combined therapy: split dose (Group C)*

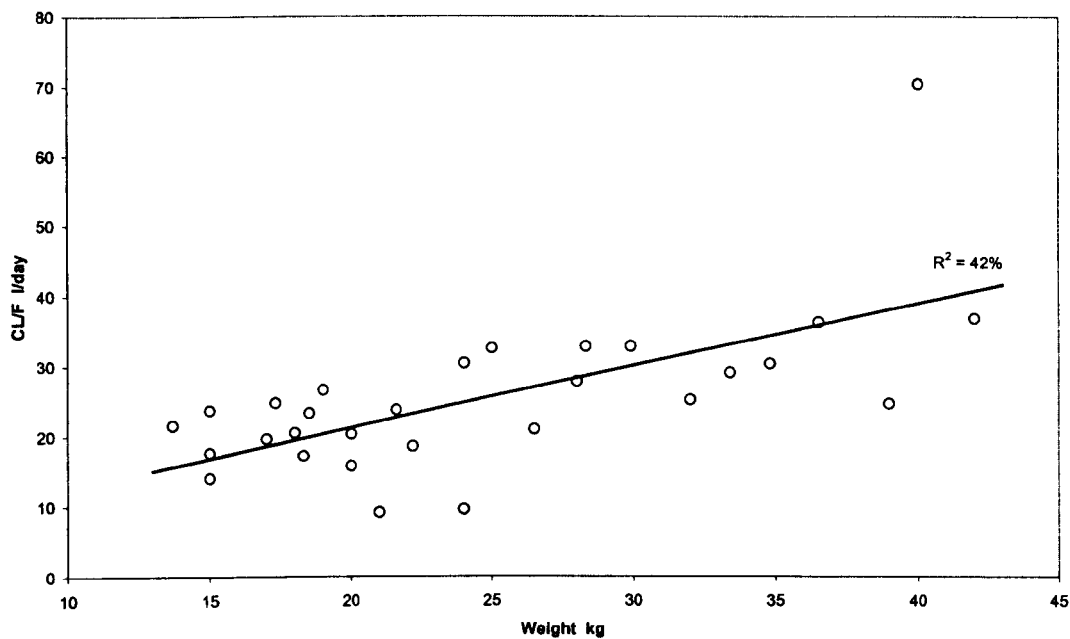
The posterior patient specific estimates for CL/F and V/F were significantly positively correlated with body weight (Figures 3.6a & 3.6b). The incorporation of body weight in the model as a covariate for both CL/F and V/F resulted in a reduction in the inter-patient variability from 48 to 37% for CL/F and from 39 to 26% for V/F. The minimum, mean and maximum admission body weights were 14, 24 and 42 kg respectively. At these body weights the respective population estimates of CL/F would be 15.27, 23.77 and 39.07 l/day, and for V/F would be 211.53, 330.63 and 545.01 l. The final model parameter estimates are given in Table 3.6. These results confirm that the dose recommendation for split dose mefloquine should be adjusted for body weight.

#### *Mefloquine combined therapy: single dose (group D)*

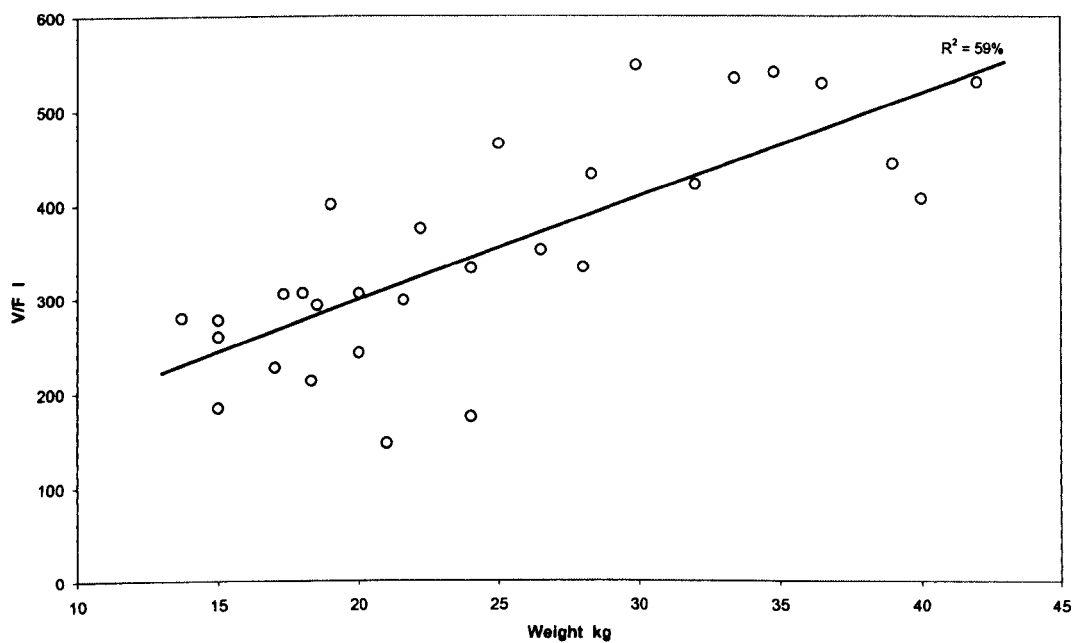
The posterior patient specific estimates for CL/F and V/F were significantly positively correlated with body weight (Figures 3.6c & 3.6d). Incorporating body weight as a covariate for both CL/F and V/F in the model reduced the inter-patient variability from 66 to 49% for CL/F and from 50 to 34% for V/F. At the minimum (13 kg), mean (25.6 kg) and maximum (43 kg) body weights the respective population estimates of CL/F would be

13.34, 33.0 and 60.14 l/day, and for V/F would be 192.34, 383.86 and 648.34 l. The final model parameter estimates are given in Table 3.6. These results confirm that the dose recommendation for single dose mefloquine should be adjusted for body weight.

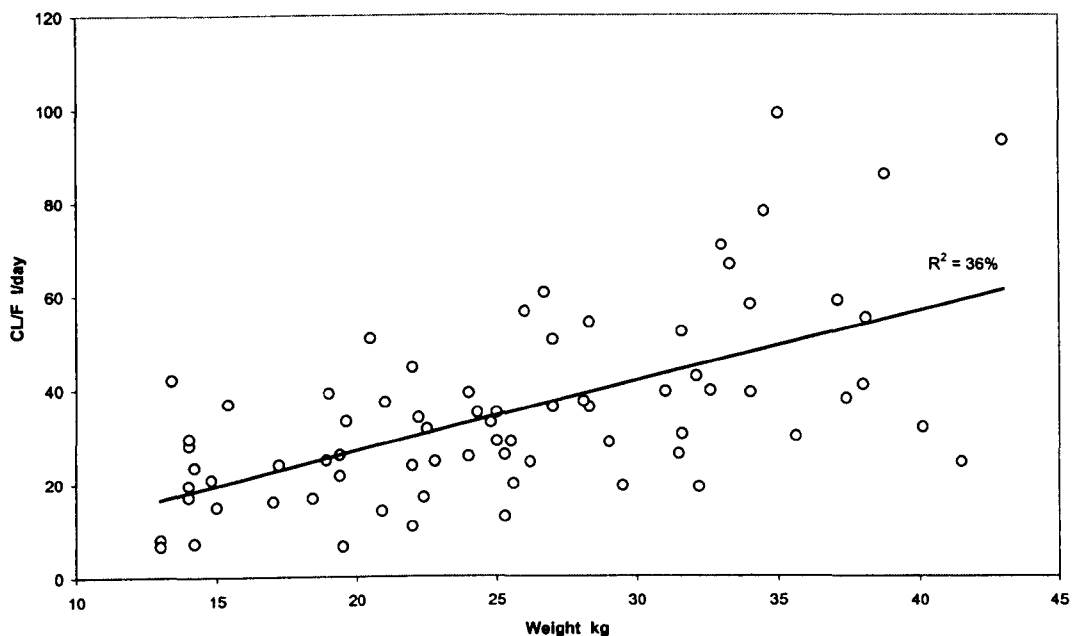




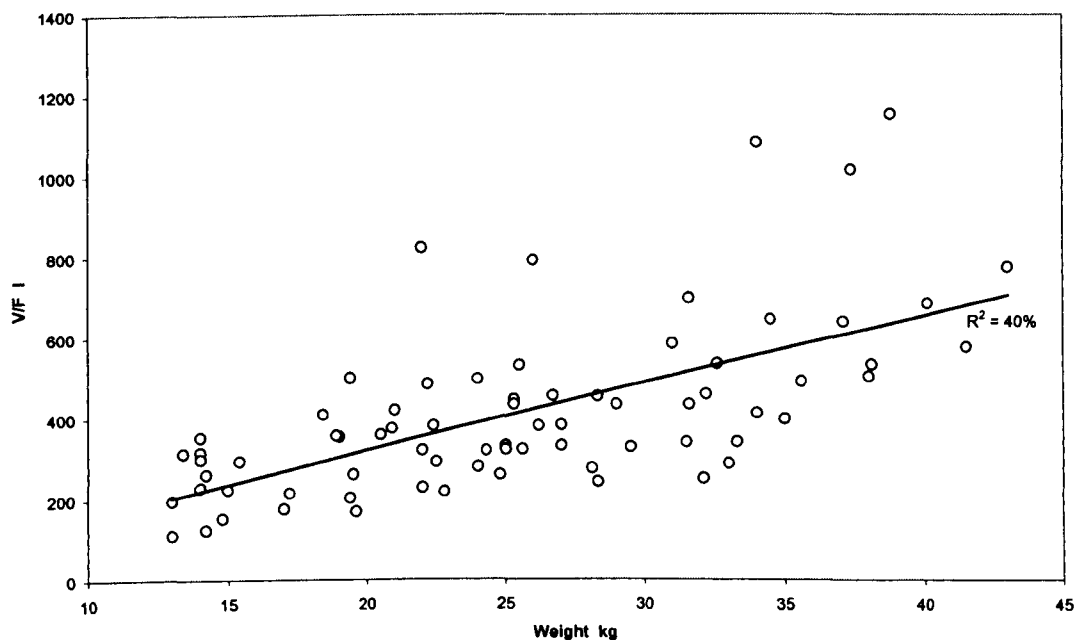
**Figure 3.6a.** Relationship between posterior estimates of CL/F (l/day) and weight (kg) in patients from group C (mefloquine & artesunate: split dosing). The line of best fit is also shown.



**Figure 3.6b.** Relationship between posterior estimates of V/F (l) and weight (kg) in patients from group C (mefloquine & artesunate: split dosing). The line of best fit is also shown.



**Figure 3.6c.** Relationship between posterior estimates of CL/F (l/day) and weight (kg) in patients from group **D** (mefloquine & artesunate: single dosing).  
The line of best fit is also shown.



**Figure 3.6d.** Relationship between posterior estimates of V/F (l) and weight (kg) in patients from group **D** (mefloquine & artesunate: single dosing).  
The line of best fit is also shown.

**Table 3.6. Mefloquine population pharmacokinetic parameters after adjusting for body weight.**

	Combined therapy & split dosing.		Combined therapy & single dosing.	
	Estimate (SE)	90%	Estimate (SE)	90%
		Prediction		Prediction
		Intervals.		Intervals.
	Group C		Group D	
CL/F (l/day) *	23.77 (2.16)	12.87, 43.90	33.00 (2.40)	14.86, 73.28
Change in CL/F (l/day) due to an increase of 1 kg in body weight	0.85 (0.29)		1.56 (0.26)	
V/F (l) *	330.63 (19.19)	217.35, 502.94	383.86 (18.27)	220.50, 668.24
Change in V/F (l) due to an increase of 1 kg in body weight	11.91 (2.65)		15.20 (2.10)	
k <sub>e</sub> (elimination rate /day)*	0.072 /day		0.086 /day	
t <sub>1/2</sub> (elimination half life – days) *	9.63 days		8.06 days	
σ <sub>e</sub> (ng/ml)	259.2		295.6	

\* - For a person having a mean body weight (i.e. 24.0 kgs for Group C and 25.6 kgs for Group D)

### 3.3.6 Relationship between pharmacokinetic parameters and therapeutic response

All but one of the patients treated with mefloquine combined with artesunate cleared their parasitaemias within the first 48 hours. Patients treated with the combination also had a very low failure rate; 2% (defined as a recrudescence infection within 28 days). This contrasts with patients treated with mefloquine alone; only 37% had cleared their parasitaemia within the first 48 hours, and the 28 day failure rate was 24% (Table 3.7). The addition of artesunate therefore improved cure rates considerably, and thus obscured the relationship between mefloquine blood concentration profiles and the therapeutic response. Therefore to investigate the association between PK parameters and therapeutic response, only patients who received mefloquine monotherapy (groups A & B, n=152) were included in this analysis.

**Table 3.7. Comparison of therapeutic response between mefloquine monotherapy and combined treatment with artesunate.**

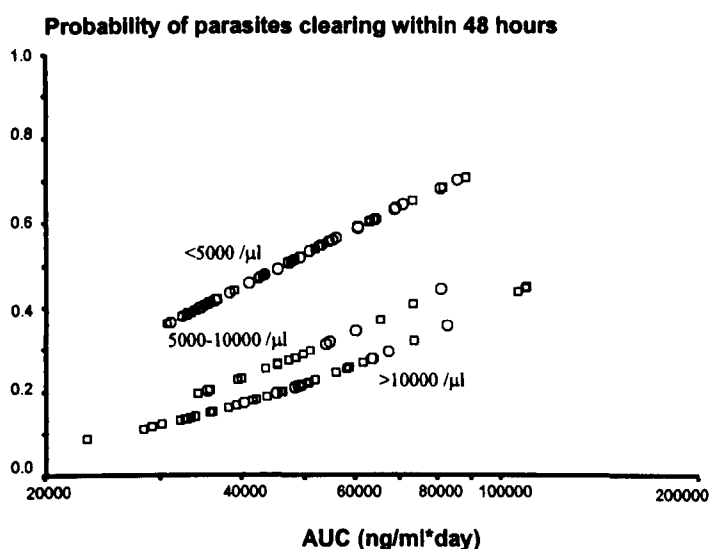
	Mefloquine alone	Mefloquine + Artesunate	P-value*
Parasites cleared within 24 hrs	14.8% (18/122)	77.4% (72/93)	<0.001
Parasites cleared within 48 hrs	36.8% (43/117)	99.0% (95/96)	<0.001
Fever cleared within 24 hrs	65.6% (61/93)	50.0% (20/40)	0.091
Fever cleared within 48 hrs	84.3% (75/89)	100% (20/20)	0.100
Failed within 28 days	24.1% (33/137)	2.0% (2/98)	<0.001

\* - Chi squared statistic with Yates' correction

#### *Mefloquine monotherapy (groups A & B)*

Patients who had cleared all their parasites within the first 48 hours had a higher AUC<sub>0-∞</sub> value compared to those patients with late parasite clearance, although this difference was only of borderline significance (geometric mean (95% CI, n): 50373 (46121-55017, 43) vs 45583 (42306-49125, 74) ng/ml\*day, p=0.05). From the multiple logistic regression analysis with adjustment for age, type of dosage (split versus single), admission parasitaemia and temperature, a higher mefloquine AUC<sub>0-∞</sub> (p=0.038) was associated with

a significantly increased probability of parasite clearance within 48 hours (Figure 3.7). Patients still febrile after 24 hours had a significantly lower  $AUC_{0-\infty}$  value compared to patients whose fever had cleared within one day (geometric mean (95% CI, n): 43561 (39003 - 48652, n=32) vs 50874 (46903 - 55170, n=61) ng/ml\*day,  $p=0.026$ ). Similar findings were observed for fever clearance at 48 hours, although the relationship between fever clearance in the first 24 or 48 hours and  $AUC_{0-\infty}$  values no longer remained after adjustment for the confounders mentioned above. There was no difference in  $AUC_{0-\infty}$  and V/F values between those patients whose infection recrudesced within 28 days and those who were cured.



**Figure 3.7.** Relationship of estimated probability of parasites clearing within 48 hours and whole blood mefloquine area under the concentration-time curve [ $AUC(0-\infty)$ ] values (ng/ml \*day) derived from a multiple logistic regression model.

*Top, middle and bottom fitted curves correspond to admission parasite counts in the categories: <5000/ $\mu$ l, 5000 to 10,000 / $\mu$ l, and >10,000 / $\mu$ l.*

Open circles – cleared parasites; Open squares – parasites not cleared by 48 hours.

### 3.4 Discussion

Conventional PK studies of the antimalarial, mefloquine, have observed considerable inter-individual variability in the PK parameters. The development of a population PK model for mefloquine provides estimates of the PK parameters, and an assessment of the different sources (e.g. clinical, demographic or laboratory factors) of inter-individual variance in these parameters.

#### 3.4.1 Summary of findings

In this study of mefloquine blood concentrations obtained prospectively over a five year period from patients with multi-drug resistant falciparum malaria, the final population PK estimates were similar to those reported from previous studies (Karbwang and White, 1990). Estimates were obtained for inter-individual variation in apparent clearance and volume of distribution. In these studies the fraction of drug absorbed (F) is not known, therefore an increase in estimated  $CL/F$  or  $V/F$  could result either from an increase in clearance or volume, or a reduction in F. As there is no reason to believe that the method of dosing should alter clearance or volume, differences in these parameters between dosage regimens were attributed to variation in the fraction of the drug absorbed. Splitting the 25mg/kg dose of mefloquine monotherapy was associated with a 50% increase in oral bioavailability, and for the combination treatment, bioavailability increased by 20%. This finding provides further support for this method of administration since previous studies have shown that splitting the dose of mefloquine into 15 and 10 mg/kg (at least 8 hrs apart) halves the rate of early vomiting (ter Kuile et al. 1995a), and vomiting has been shown to be a risk factor for treatment failure (ter Kuile et al. 1995b). There are two possible explanations for the increase in oral bioavailability for patients receiving the split dose regimen; a disease related reduction in oral bioavailability or that the absorption of

mefloquine in malaria is capacity limited. The observation that splitting the dose of mefloquine produces a much smaller increase in bioavailability when given in association with an artemisinin derivative argues in favour of a disease related reduction in oral bioavailability since artesunate accelerates recovery from malaria. This is further supported by the larger  $AUC_{0-\infty}$  found when the single dose of mefloquine in combined treatment was delayed until the second day of treatment. Acute falciparum malaria is associated with altered gastrointestinal motility, reduced visceral perfusion, and impaired absorption. Splitting the dose of mefloquine ensures that the second oral dose is given when the patient is in an improved clinical state.

Mefloquine PK were influenced relatively little, or not at all, by factors such as age, weight, sex, or measures of acute malaria severity. It should be noted that only children aged 5 to 14 years were included in the studies where mefloquine was given in combination with artesunate, whereas patients treated with mefloquine monotherapy varied from 1 to 54 years of age. The lack of an association between age and the PK of mefloquine monotherapy suggests that no dosage adjustment is required for children. The original decision to adjust the dose of mefloquine according to body weight was an empirical decision that had not been tested formally. In this chapter the association between mefloquine PK and body weight for both actual dose (mg) and weight adjusted dose (mg/kg) was explored. For actual dose (mg), there were strong linear positive associations between the PK parameters (clearance and volume) and body weight. For weight adjusted dose (mg/kg), there were no associations between the PK parameters and body weight. The therapeutic implications of these data are that mefloquine dosage per kg body weight is a good approach and need not be further adjusted. Note the patients in the current dataset have body weights varying from 8 to 61 kgs. Therefore the observed findings may not hold for larger adults or small babies.

Whilst demographic factors could alter drug elimination, disease related factors are unlikely to have a major effect on the terminal phase of elimination once the patient has recovered from malaria (usually within 3 or 4 days). However in the acute phase of malaria, in addition to absorption alterations, there may be changes in volume of distribution related to altered tissue and blood drug binding, and changes in clearance related to impaired hepatic function and, for mefloquine, interruption in the entero-hepatic cycling of the drug. The shorter terminal half-life of mefloquine in patients with malaria compared to volunteers (Palmer et al. 1993) has been attributed to interruption in entero-hepatic recycling. Body temperature on admission was found to affect mefloquine blood concentrations in patients who received mefloquine monotherapy and split dosing, but this was not a significant covariate in the other patient groups. The relationship between body temperature on admission and  $V/F$  is complicated because some of the calculation of  $V/F$  is derived from data collected after fever clearance. If  $V/F$  changes then either  $V$  changes and/or  $F$  changes. From these data and since there was no association between temperature on admission and  $CL/F$  it is difficult to say whether body temperature on admission changes  $V$  or  $F$ .

Despite considerable inter-patient variability in whole blood mefloquine concentration profiles, the derived PK parameters were not related significantly to the probability of cure. But as there were only 33 recrudescences in the 137 patients with mefloquine monotherapy (15 of the patients dropped out of the study before 28 days), this large PK study had limited power to detect such associations. These observations contrast with observations of halofantrine and lumefantrine (ter Kuile et al. 1993b; White et al. 1999a). Absorption of these two antimalarials is very variable. Plasma concentration profiles (particularly the  $AUC_{0-\infty}$  or the day 7 blood concentration) vary even more than for mefloquine, and are



major determinants of therapeutic outcome. The data with mefloquine suggest that, in this series, the variation in the infecting parasites' intrinsic drug sensitivity, and the immune responses of the patient which synergise with the antimalarial drug, were relatively more important than the variation in the patients' mefloquine blood concentration profiles in determining treatment response. However it is evident, from the considerably better cure rates with high dose (25 mg/kg), compared to low dose (15 mg/kg), that the profiles of blood mefloquine concentrations are an important determinant of the therapeutic response (ter Kuile et al. 1992).

The improvements in oral bioavailability associated with splitting the mefloquine dose resulted in higher blood concentrations. This was associated with more rapid parasite clearance, and this has been shown to correlate with cure in mefloquine treated patients (ter Kuile et al. 1992; ter Kuile et al. 1995b). This suggests that cure rates would be improved by splitting the mefloquine dose. Nevertheless there was still considerable inter-individual variance in PK with split-dosing which suggests that the absorption of the drug was still far from complete. These data suggest that high dose mefloquine should always be given in a split dosage to optimise absorption and minimise treatment failures.

#### 3.4.2 Limitations of the data

It was not possible to estimate the absorption rate constant ( $k_a$ ) nor the inter-individual error of  $k_a$  from the data available as no blood samples were collected within 24 hours following drug administration. The patients treated with mefloquine were suffering from uncomplicated *P. falciparum* malaria and not severe malaria. Therefore there was no clinical benefit to the patient to collect blood samples during the absorption phase of the drug. Since  $k_a$  could not be estimated from the data for the above reasons,  $k_a$  was fixed at a value of 7 /day which was obtained from a different study of the same population (Nosten

et al. 1991a). The influence of fixing  $k_a$  on subsequent parameter estimation has been comprehensively investigated by Wade et al. (1993). If no data are present in the absorption phase, as is the case with the mefloquine data, then misspecifying the rate of drug absorption and the model used to describe drug absorption (first-order absorption was chosen for the mefloquine data) has little consequence for estimation of the remaining population parameters. However if data contain even the slightest information about absorption, then a parameter to describe inter-subject variability in absorption should be included to protect against the inter-subject variability in  $k_a$  being manifested into the inter-subject variability of apparent volume of distribution.

Other investigators have fitted two compartment models to characterise the PK of mefloquine (Looareesuwan et al. 1987; Boudreau et al. 1990). With two compartment models the drug has two decline phases, commonly a rapid phase followed by a slower phase. The data analysed in this chapter had blood samples only collected up to day 28 while Looareesuwan et al. (1987) and Boudreau et al. (1990) collected blood samples up to days 63 and 67 respectively. In the paper by Looareesuwan et al. (1987) the terminal half-life was observed to be 10 to 11 days, but no value was published for the first half-life. Boudreau et al. (1990) presented distributions of the elimination half-lives of four groups: cured ( $n=10$ ) and recrudescant patients ( $n=6$ ) treated with 750 mg of mefloquine, and cured ( $n=9$ ) and recrudescant patients ( $n=2$ ) treated with 1500 mg of mefloquine. The mean half-life of the first phase varied from two to five days and the mean half-life of the second phase varied from 19 to 45 days for the different groups of patients described above. Visual inspection of the PK profiles published by Boudreau et al. (1990) suggest a crossover between the fast and slow phases at around 10 to 12 days. The data presented in this chapter had only five to six main time points where samples were collected to form the population PK model. For the second phase, which occurs approximately 10 days after

drug administration, samples were collected before 1994 on days 14, 21 and 28 and after 1994 on only days 14 and 28. Thus there is little spread in the sampling times for a second phase to be adequately characterised. In other words the failure in the present dataset to define a two compartment model could be due to the lack of a wide spread of sampling times. Kowalski and Hutmacher (2001) found from a simulation study that a one-compartment model approximation to the plasma concentration-time curve for a drug known to exhibit two-compartment kinetics yielded accurate mean estimates of volume and clearance as well as subpopulation differences in clearance.

## 4. MEFLOQUINE PHARMACOKINETIC-PHARMACODYNAMIC MODELS

### 4.1 Introduction

In this chapter a mechanistic mathematical model was developed to describe the change in total parasite burden with time *in vivo*, in the presence of drug. The mathematical model was able to predict the total elimination of parasites, that is, radical cure. Population pharmacodynamic modelling of *in vitro* concentration-effect curves was performed to obtain estimates for some of the parameters of the mechanistic mathematical model. Total parasite burden versus time profiles *in vivo* were simulated for different scenarios to explore the influence of the pharmacokinetic (PK) and pharmacodynamic (PD) parameters on treatment cure.

### 4.2 Methods

#### 4.2.1 Mathematical Model

To find a mathematical equation that describes the change in total parasite burden over time in the presence of the drug, a slightly altered form of the mathematical model published by Hoshen et al. (1998), has been used.

The number of parasites in a patient at any time ( $P_t$ ) is a balance between their average multiplication rate and the proportion that are killed and cleared by the antimalarial and host defences. Thus the change in parasitaemia can be described as :-

$$\frac{dP}{dt} = a.P - f(C).P - f(I).P$$

where ‘ $a$ ’ is a parameter representing the growth rate constant of the parasite;

$f(C)$  is a function, dependent on the concentration of the drug ( $C$ ), that represents the killing of the parasites; and  $f(I)$  is a function that represents the hosts’ background immunity to malaria.

The host contribution, which reflects “background immunity”, contributes relatively little to drug effect at high concentrations, but becomes important at low drug concentrations. It will not be used further in this analysis, although in areas of high stable transmission where partially effective or ineffective drugs are used it is the main determinant of therapeutic outcome.

Thus

$$\frac{dP}{dt} = a.P - f(C).P$$

Killing is considered a first order process which continues unchanged throughout the process of parasite elimination (Day et al. 1996). It should be noted that malaria is exclusively an intravascular infection (extravascular forms are not pathogenic) and that the antimalarial activity is related to plasma concentrations of free (unbound) antimalarial drug.

Parasite killing can then be described by an  $E_{\max}$  type model.

$$f(C) = k_1 * \frac{C^\gamma}{C^\gamma + C_{50}^\gamma}$$

where  $k_1$  - rate constant of parasite killing,

$\gamma$  - slope of concentration effect curve,

$C$  – antimalarial drug concentration,

&  $C_{50}$  – drug concentration which kills 50% of the parasites.

The plasma antimalarial drug concentration *in vivo* is not constant; it rises as the drug is absorbed and then declines over time as the drug distributes throughout the body and the drug is eventually eliminated. In the case of mefloquine the elimination phase has been shown to be biexponential or triexponential (White, 1992c). Assuming that the intermediate concentrations which are selective occur only during the terminal phase (at least at low or intermediate levels of resistance), a monoexponential elimination phase corresponding to the slower second elimination phase of the drug was utilised.

Therefore:-

$$C = C_0 * e^{-k.t}$$

where  $t$  – time (days),

$k$  – terminal elimination rate constant of mefloquine,

&  $C_0$  – maximum concentration of mefloquine (i.e.  $C_{\max}$ ).

$P_t$  was determined by integrating  $\frac{dP}{dt}$  (see Appendix III).

The following solution results:-

$$P_t = P_0 * e^{a.t} * \left[ \frac{(C_{50})^\gamma + (C_0 \cdot e^{-k.t})^\gamma}{(C_{50})^\gamma + (C_0)^\gamma} \right]^{k1/(k.\gamma)}$$

where  $P_0$  = total parasite burden at time 0 (i.e. before administration of mefloquine).

#### 4.2.2 Parameter estimation and statistical analysis

In non-immune subjects, the multiplication rate of the asexual stages of *P. falciparum* in the blood averages six-fold and can reach 20-fold per cycle (Kitchen, 1941; Kitchen, 1949). For the model predictions presented in the results section (4.3) 'a' was calculated assuming that a single asexual malaria parasite multiplies 10 fold every two days. This gives a growth rate constant 'a' of 1.15 (ln10 /2). The terminal elimination rate constant (k) of the drug was assumed to be 0.036 (/day) (that is the value derived from population PK modelling of patients receiving a single dose of 25 mg/kg of mefloquine monotherapy; Chapter 3, Table 3.2), following a treatment dose of mefloquine. The maximum parasite killing rate resulting from mefloquine was assumed to be 99% per cycle or 90% / day which corresponds to  $k_1=3.45$  /day. This corresponds to a parasite reduction ratio (PRR) of 100 fold, a relatively poor antimalarial effect, but one that has been documented, and is associated with mefloquine resistance (ter Kuile et al. 1992; White, 1999b). Killing rates up to 99.9% per cycle may occur with mefloquine. The artemisinin derivatives have been shown to have the highest PRRs of all the antimalarial drugs, greater than 10,000 or a reduction of 99.99% parasites per two day asexual life cycle (White, 1997).

As the mutational events which may confer resistance occur randomly, resistant mutants are more likely to occur in high biomass infections. Therefore a "worst case scenario" has been chosen of a relatively high biomass *P. falciparum* infection corresponding to a total parasite burden at time zero of  $10^{12}$  parasites in an adult (which corresponds to a parasite

count of approximately 100,000 / $\mu$ l or 2% parasitaemia). The parameter estimates chosen above do not represent an “average patient”, but instead a patient with a relatively high parasite burden from an area with existing mefloquine resistance.

For the parameter  $C_0$  the value of 1200 ng/ml was chosen which was derived from the population PK modelling of data from 24 patients treated with single dose of 25 mg/kg of mefloquine monotherapy (Chapter 3). The parameter estimates and their respective variances from the derived population PK model (Table 4.1) were also used to simulate 1000 PK profiles. The distribution of the inter-subject variability of the pharmacokinetic parameters was lognormal while the residual error was normally distributed (Table 4.1). The inter-subject variability values for the different PK parameters were assumed to be independent of each other. The 1000 simulations were performed using the computing package QBASIC. These simulated profiles were used to obtain estimates of the lowest and highest mefloquine concentrations that would be achieved in a population.

**Table 4.1:- Parameters used for simulation of pharmacokinetic profiles**

Parameter	Estimate (SE)	Estimate <sup>^</sup>
CL/F (l/kg/day)	0.733 (0.051)	0.704
V/F (l/kg)	20.37 (2.53)	20.56
$\omega_{CL/F}$	0.15	0.16
$\omega_{V/F}$	0.56	0.55
Residual additive error - $\sigma_e$ (ng/ml)	180.4	

CL/F - clearance divided by the fraction of drug absorbed;

V/F - total apparent volume of distribution divided by the fraction of drug absorbed;

$\omega$ - inter-subject variability in CL/F and V/F, modelled using multiplicative error models;

<sup>^</sup> - estimates obtained from non-linear mixed effects modelling of the 1000 simulated pharmacokinetic profiles.



Assuming one schizont releases 10 merozoites then the minimum inhibitory concentration (*MIC*) at which the net parasite multiplication rate (*PMR*) is unity corresponds approximately to the *EC*<sub>90</sub> (i.e. the concentration which is 90% effective).

Now if the *MIC* is the concentration at which the total parasite burden is not changing (i.e. a transient steady state) then:

$$\frac{dP}{dt} = a.P - f(MIC).P = 0$$

Substituting for  $f(MIC)$  :-

$$a - k_1 * \left( \frac{MIC^\gamma}{MIC^\gamma + C_{50}^\gamma} \right) = 0$$

$$C_{50} = \left[ \frac{(k_1 - a) * MIC^\gamma}{a} \right]^{1/\gamma}$$

Assuming  $MIC \approx EC_{90}$ :-

$$C_{50} = \left[ \frac{k_1 - a}{a} \right]^{1/\gamma} * EC_{90}$$

The *EC*<sub>90</sub> *in vivo* is unknown but it must be related to the *EC*<sub>90</sub> *in vitro*. However because *in vitro* susceptibility assessments are conducted in the absence of plasma (to which mefloquine is reported to bind avidly), platelets and white cells, the two are not equivalent. Unfortunately because mefloquine also binds to laboratory ware, notably plastic, precise estimates of plasma protein binding are not available.

Thus,

$$EC_{90} (in vivo) = m * EC_{90} (in vitro)$$

[where  $m$  is an unknown parameter]

For the simulations presented in the results section of this chapter  $m$  was given the value of 10. This value was chosen empirically. The  $EC_{90} (in vitro)$  was derived from modelling of *in vitro* concentration-effect curves where the effect measure is inhibition of parasite uptake of [ $^3$ H] hypoxanthine. It was assumed that the slope ( $\gamma$ ) of the concentration-effect curve *in vivo* is the same as that observed *in vitro*. For the parameters  $\gamma$  and  $EC_{90}$ , population estimates were derived from non-linear mixed effects modelling of *in vitro* concentration-effect curves of mefloquine (Lindstrom & Bates, 1990). Non-linear mixed effects models produce parameter estimates for the complete drug population and posterior estimates for each infecting isolate. The dataset contained a total of 415 *in vitro* concentration-effect curves from *P. falciparum* isolates collected by three different laboratories based in Thailand.

#### *In vitro* susceptibility testing:

All laboratories used a semi-automated dose response assay as described by Desjardins et al. (1979), and Webster et al. (1985), and used the same beta counter. Growth of synchronous parasite isolates or cultures was estimated using parasite uptake of a radioactive DNA precursor, [ $^3$ H] hypoxanthine, as a measure. Percentage growth at different mefloquine concentrations was calculated as the proportion of the counts in the drug free wells. Background counts (unparasitised, drug free wells) were subtracted from all counts.

The Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok, Thailand measured 345 curves. The samples used in the dose response assays were collected in field trials conducted throughout Thailand between 1990 and 1994.

The Wellcome Unit, Bangkok measured 44 dose response curves. The samples were collected during 1997 and 1998 from the Hospital for Tropical Diseases, Bangkok and on the western border of Thailand.

The Shoklo Malaria Research Unit (SMRU) measured 26 dose response curves. These were collected from refugee camps situated along the western border of Thailand. The 415 isolates comprise a wide range of mefloquine sensitivities from fully sensitive to highly resistant, which allow estimates of the best and worst possible scenarios for  $\gamma$  and  $EC_{90}$ .

The PD model fitted was the following sigmoid inhibitory effect model:-

$$E = E_{\max} - (E_{\max} - E_0) * \left[ \frac{C^\gamma}{C^\gamma + (EC_{90}^\gamma / 9)} \right]$$

This is a reparameterised version of the standard sigmoid inhibitory effect model where  $EC_{50}$  equals  $EC_{90} / 9^{1/\gamma}$ .  $E$  represents the proportion of uptake of [ $^3\text{H}$ ] hypoxanthine in the nucleoprotein of the parasites in drug-free control wells corresponding to unrestrained growth *in vitro*. Maximum effect occurs when there is no uptake of [ $^3\text{H}$ ] hypoxanthine (no growth).  $E_0$  represents minimum percent growth,  $E_{\max}$  is the maximum percent growth,  $EC_{90}$  is the concentration of mefloquine required to inhibit 90 percent of the control parasites' hypoxanthine uptake and  $\gamma$  is the slope of the curve. It should be noted that the exact relationship between inhibition of hypoxanthine uptake and growth inhibition has not been characterised.

Inter-strain variability in  $E_{\max}$ ,  $E_0$ ,  $\gamma$  and  $EC_{90}$  were modelled with multiplicative error models. Multiplicative error models ensure the individual strain parameters (e.g.  $E_{\max,i}$ ) must be greater than zero and that their distribution is skewed to the right.

$$E_{\max,i} = E_{\max} \cdot \exp(\eta_i^{E_{\max}})$$

$$E_{0,i} = E_0 \cdot \exp(\eta_i^{E_0})$$

$$\gamma_i = \gamma \cdot \exp(\eta_i^{\gamma})$$

$$EC_{90,i} = EC_{90} \cdot \exp(\eta_i^{EC_{90}})$$

The inter-strain variability ( $\eta$ ) values for the above four parameters were assumed to be independent of each other.

The residual intra-strain error was modelled using an additive error model since the error between the observed and predicted values was observed to be constant.

$$E_{ij} = E_{pij} + \epsilon_{ij}$$

$E_{\max,i}$ ,  $E_{0,i}$ ,  $\gamma_i$  and  $EC_{90,i}$  are the PD parameters for the isolate and  $E_{\max}$ ,  $E_0$ ,  $\gamma$  and  $EC_{90}$  are the population means. The  $\eta_i^{E_{\max}}$ ,  $\eta_i^{E_0}$ ,  $\eta_i^{\gamma}$  and  $\eta_i^{EC_{90}}$  are random effects with zero mean and variances  $\sigma^2_{E_{\max}}$ ,  $\sigma^2_{E_0}$ ,  $\sigma^2_{\gamma}$  and  $\sigma^2_{EC_{90}}$ , respectively. They represent the inter-isolate variability for each of the parameters. The magnitude of this variability is expressed as a coefficient of variation (CV).  $E_{ij}$  is the  $j^{\text{th}}$  measure of effect for the  $i^{\text{th}}$  strain and  $E_{pij}$  is the  $j^{\text{th}}$  predicted measure of effect for the  $i^{\text{th}}$  strain.  $\epsilon_{ij}$  is the residual intra-subject error term and is assumed to be randomly and normally distributed with mean of zero and variance  $\sigma^2$ . The NLME procedure (Lindstrom and Bates, 1990) of the SPLUS data programme (SPLUS 4.5 for Windows, Mathsoft, Inc.) was used to calculate estimates of the population PD parameters ( $E_{\max}$ ,  $E_0$ ,  $\gamma$ ,  $EC_{90}$ ) and their respective inter-strain variances ( $\eta^{E_{\max}}$ ,  $\eta^{E_0}$ ,  $\eta^{\gamma}$ ,  $\eta^{EC_{90}}$ ). The programme also provides estimates of the residual random intra-strain error ( $\epsilon$ ) and the variances of the inter-strain error terms ( $\sigma^2_{E_{\max}}$ ,  $\sigma^2_{E_0}$ ,  $\sigma^2_{\gamma}$ ,  $\sigma^2_{EC_{90}}$ ). The goodness of

fit of each model (e.g. models having different numbers of random effects) was determined by the change in the objective function (minus twice the loglikelihood of the data). A significant drop in the objective function (with use of the  $\chi^2$  distribution) determined the model that best described the data. The goodness of fit of each model was also determined by the precision of the parameter estimates and examination of the scatter plot of residuals versus predicted measure of effect.

## 4.3 Results

### 4.3.1 *In vitro* concentration-effect curves

Data collected from three different laboratories in Thailand were modelled to estimate parameters that describe the *in vitro* concentration-effect relationships for mefloquine. The model that gave the best fit to the three different sources of data was the inhibitory sigmoid  $E_{\max}$  effect model with  $E_{\max}$ ,  $EC_{90}$  and  $\gamma$  fitted as random effects. Fitting  $E_0$  as a random effect did not significantly reduce the objective function. The SPLUS programme used for fitting the sigmoid  $E_{\max}$  model is given in Appendix IV. The mean population PD parameters of the final model for the isolates from the three laboratories were similar (Table 4.2). These data were then combined and the sigmoid  $E_{\max}$  model fitted to all 415 isolates (Table 4.2). The population estimate of  $EC_{90}$  (population estimate = 50.4 ng/ml) had a 95% prediction interval from 213 ng/ml down to 12 ng/ml. The population estimate of the slope of the concentration-effect curve was 2.50 with the 95% prediction interval of 1.22 to 5.13. The observed and population predicted effect measures (% uptake of [ $^3$ H] hypoxanthine) versus mefloquine concentrations for the three laboratories are shown in Figures 4.1a, 4.1b and 4.1c.

**Table 4.2:- Population pharmacodynamic parameters derived from nonlinear mixed effects modelling of the *in vitro* data.**

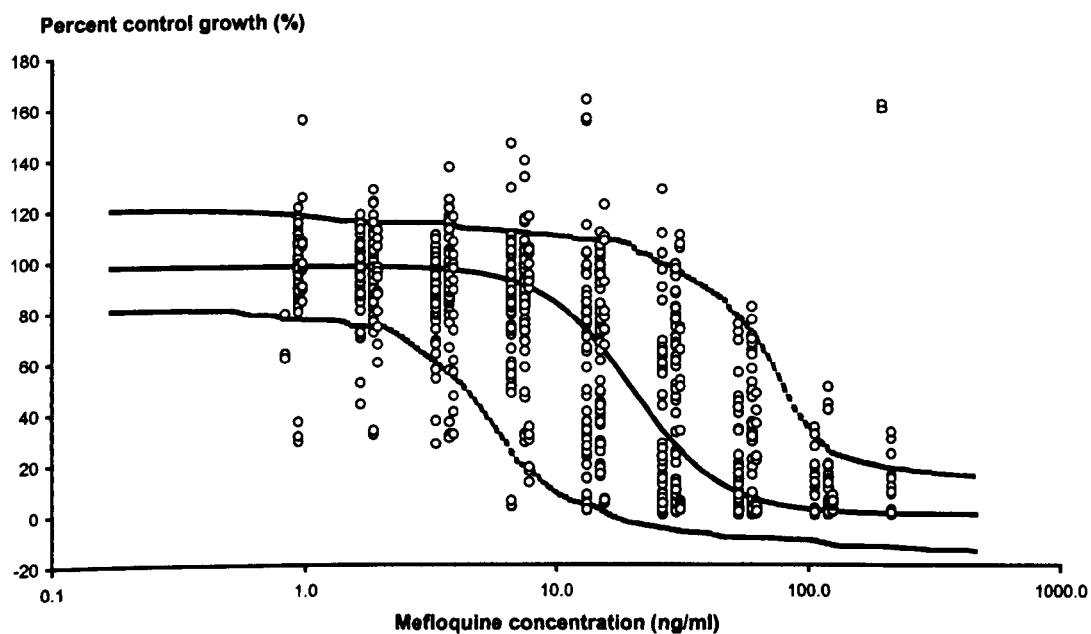
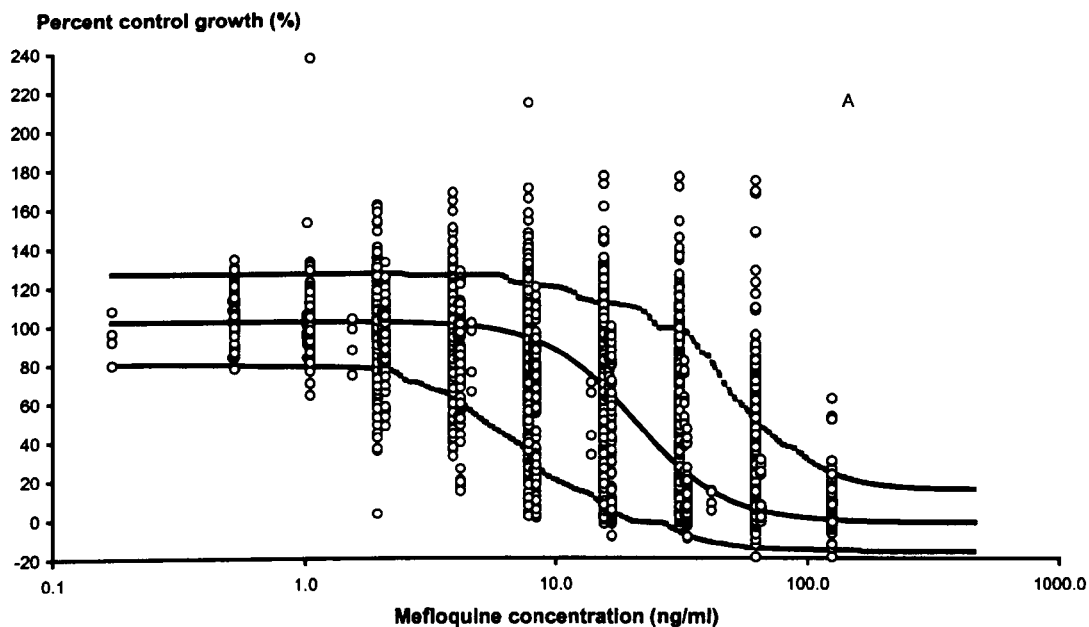
Parameter	AFRIMS	Wellcome Unit	SMRU	All 415 strains
EC <sub>90</sub> (ng/ml)	51.08 (2.20)	49.00 (6.64)	53.88 (7.49)	50.43 (1.98)
95% PI for EC <sub>90</sub> (ng/ml)	12.55 – 207.84	8.89 – 270.15	13.93 – 208.34	11.96 – 212.56
$\gamma$	2.44 (0.06)	2.47 (0.18)	3.19 (0.22)	2.50 (0.06)
95% PI for $\gamma$	1.21 – 4.92	1.03 – 5.93	1.85 – 5.49	1.22 – 5.13
E <sub>max</sub> (%)	102.30 (0.69)	97.85 (1.59)	103.96 (3.38)	101.89 (0.64)
E <sub>0</sub> (%)	-1.56 (0.46)	0.36 (0.63)	2.24 (0.69)	-0.76 (0.35)
$\omega_{E_{max}}$	0.106	0.097	0.158	0.110
$\omega_{EC_{90}}$	0.716	0.871	0.690	0.734
$\omega_{\gamma}$	0.358	0.447	0.277	0.367
$\sigma_e$ (residual error, units-%)	10.85%	8.64%	6.76%	10.31%

standard error given in parentheses; PI – Prediction Interval;

E<sub>0</sub> – minimum percent growth; E<sub>max</sub> – maximum percent growth;

EC<sub>90</sub> – concentration of mefloquine required to inhibit 90% of the control parasites' hypoxanthine uptake;  $\gamma$  - slope of the concentration effect curve;

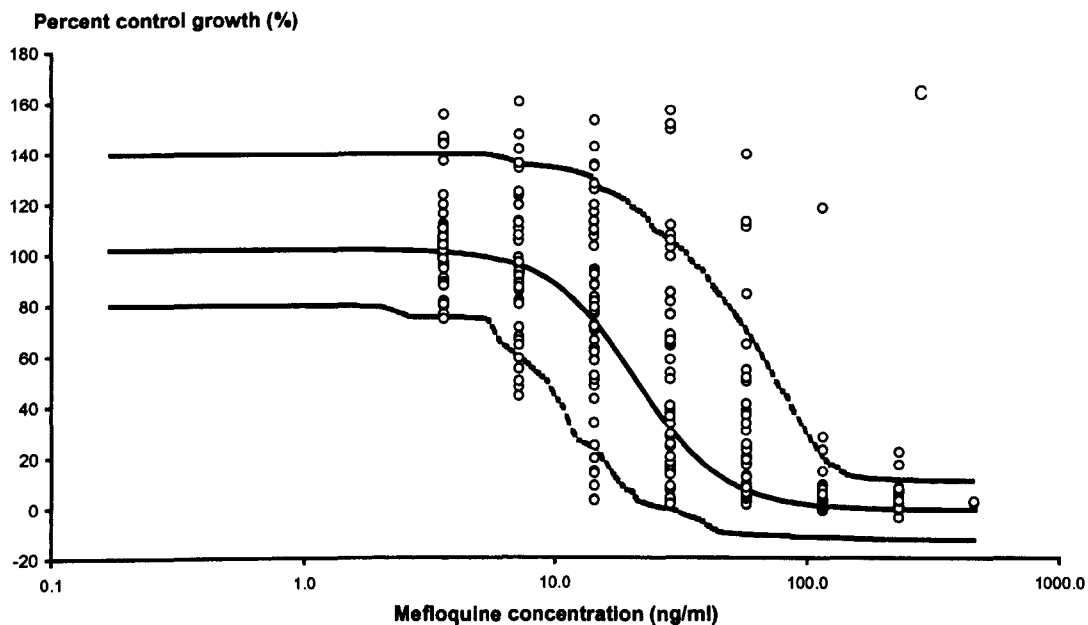
$\omega$ - inter-strain variability in E<sub>max</sub>, EC<sub>90</sub> and  $\gamma$ , modelled using multiplicative error models.



**Figures 4.1a & 4.1b.** Percent uptake of  $[^3\text{H}]$ hypoxanthine compared to that in drug-free control wells for various mefloquine concentrations. The solid line shows the fit of the data and represents the predicted concentration-time profile for the population mean. The dashed lines represent the 90% prediction intervals.

Data (A) – U.S. Armed Forces Research Institute of Medical Sciences

Data (B) – Wellcome Unit, Bangkok



**Figure 4.1c.** Percent uptake of [ $^3\text{H}$ ]hypoxanthine compared to that in drug-free control wells for various mefloquine concentrations. The solid line shows the fit of the data and represents the predicted concentration-time profile for the population mean. The dashed lines represent the 90% prediction intervals.

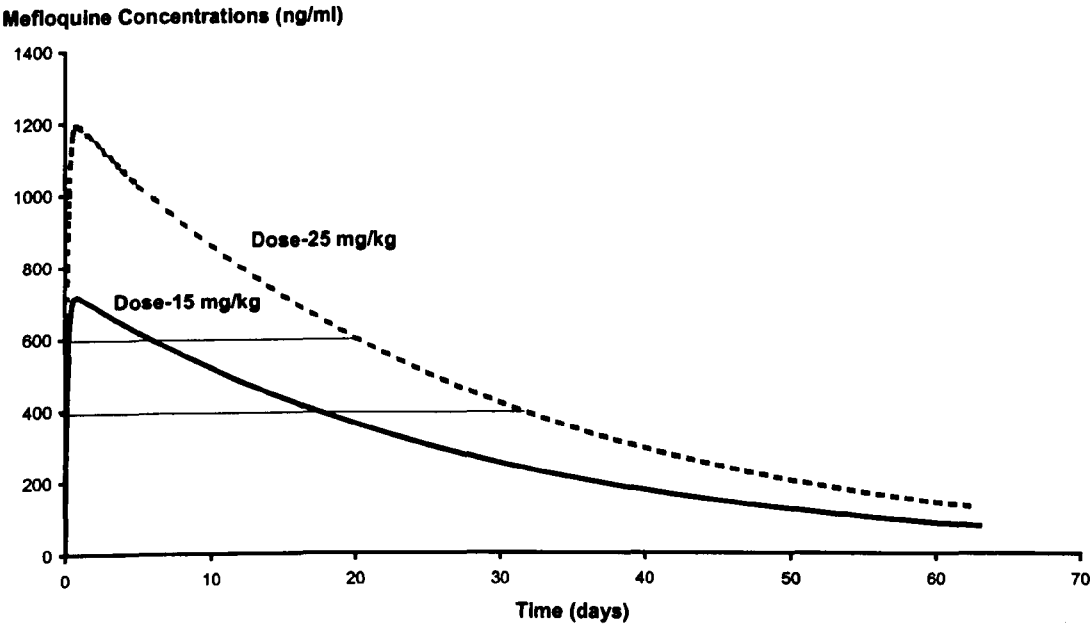
Data (C) – Shoklo Malaria Research Unit

#### 4.3.2 Pharmacokinetics of mefloquine

An important factor driving the selection of resistance is the number of parasites ( $P_t$ ) exposed to drug levels which give submaximal effects. The whole blood mefloquine concentration range giving intermediate levels of growth inhibition was chosen as 400 to 600 ng/ml. As the precise relationship between *in vivo* and *in vitro* concentration-effect relations is not known this range cannot be defined precisely. This range was chosen because in 1990, when mefloquine resistance was detected first in Thailand, the mean (95% confidence interval) serum concentration at the time of first recrudescence was 638 (546 to 730) ng/ml (Nosten et al. 1991b). This represented a concentration capable of



sustaining growth in the parasites infecting 29% of patients. A slightly lower concentration range was chosen to reflect an earlier stage of resistance. For mefloquine, the terminal elimination rate constant is dose-independent, and so the time over which the infecting parasite population is exposed to intermediate inhibitory concentrations (i.e. 400 to 600 ng/ml) is the same for different dosages of mefloquine (approximately 11 days, Figure 4.2).

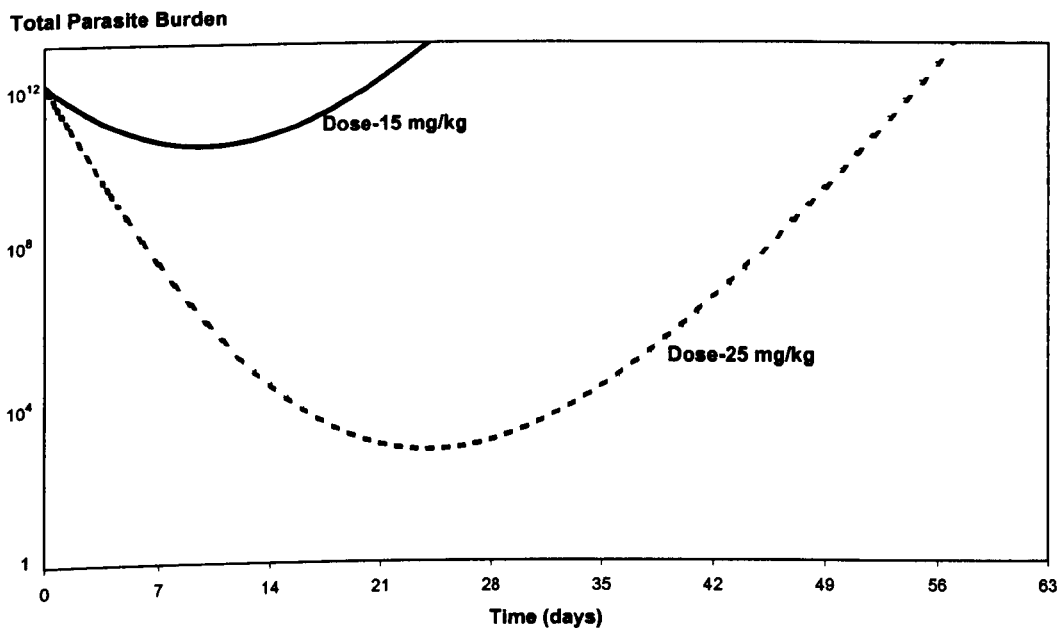


**Figure 4.2** Simulated pharmacokinetic profiles for the two standard mefloquine doses, 15 mg/kg and 25 mg/kg obtained with the parameter estimates in Table 4.1.

### 4.3.3 Predicting therapeutic outcomes

Figure 4.3 shows the model simulations of the total parasite numbers versus time for the two commonly used mefloquine doses of 15 mg/kg and 25 mg/kg, based on the parameter estimates in Table 4.3. The estimate for  $C_{50}$  is derived from the population estimates of  $EC_{90}$  and  $\gamma$ . Both  $EC_{90}$  and  $\gamma$  are the population estimates from nonlinear mixed effects modelling of the *in vitro* data of isolates from Thailand.

With the lower dose there are more parasites at all times (including during the period of intermediate or “selective” mefloquine concentrations) compared to the higher dose of 25 mg/kg. If the parasite populations are all more drug sensitive than those given in the example then the differential advantage of the higher dose is smaller - thus as resistant mutants are selected the differential advantage increases, accelerating the apparent emergence of resistance. Furthermore it is apparent that with the starting parameters chosen for the model in this case (which represented a “worse case scenario” and were consistent with a significant level of resistance), the patient will not be cured with a dose of 15 mg/kg for any admission total parasite burden since a non-immune person is only cured of malaria if the total parasite burden falls below one parasite. The same patient would be cured with a dose of 25 mg/kg if the admission total burden did not exceed  $10^9$  parasites. Obviously at lower levels of mefloquine resistance (or greater background immunity) an increasing proportion of patients treated with 15 mg/kg would be cured.



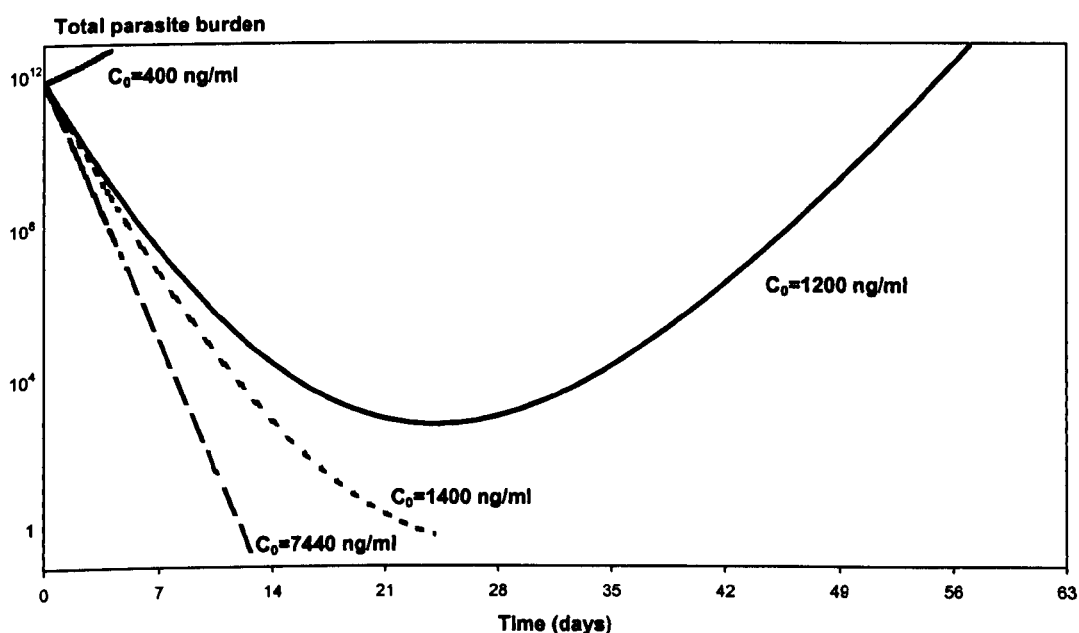
**Figure 4.3** Total malaria parasite burden versus time for the two standard mefloquine doses, 15 mg/kg and 25 mg/kg. The initial parasite burden corresponds to a parasite count of approximately 100,000 / $\mu$ l, or 2% parasitaemia in an adult with falciparum malaria.

**Table 4.3:-** Parameter estimates derived from population pharmacokinetic ( $C_0$  &  $k$ ) and pharmacodynamic ( $\gamma$  &  $C_{50}$ ) modelling and published articles ( $a$  &  $k_1$ ) (White 1997).

Parameter	Estimate
$P_0$	$10^{12}$
$a$	1.15 /day
$k$	0.036 /day
$k_1$	3.45 /day
$C_0$	1200 ng/ml (single dose of 25 mg/kg) 717 ng/ml (single dose of 15 mg/kg)
$\gamma$	2.50
$C_{50}$	665.4 ng/ml [ $m \cdot 50.43 \cdot (2)^{1/2.5}$ ] {where $m=10$ & $EC_{90}=50.43$ ng/ml}

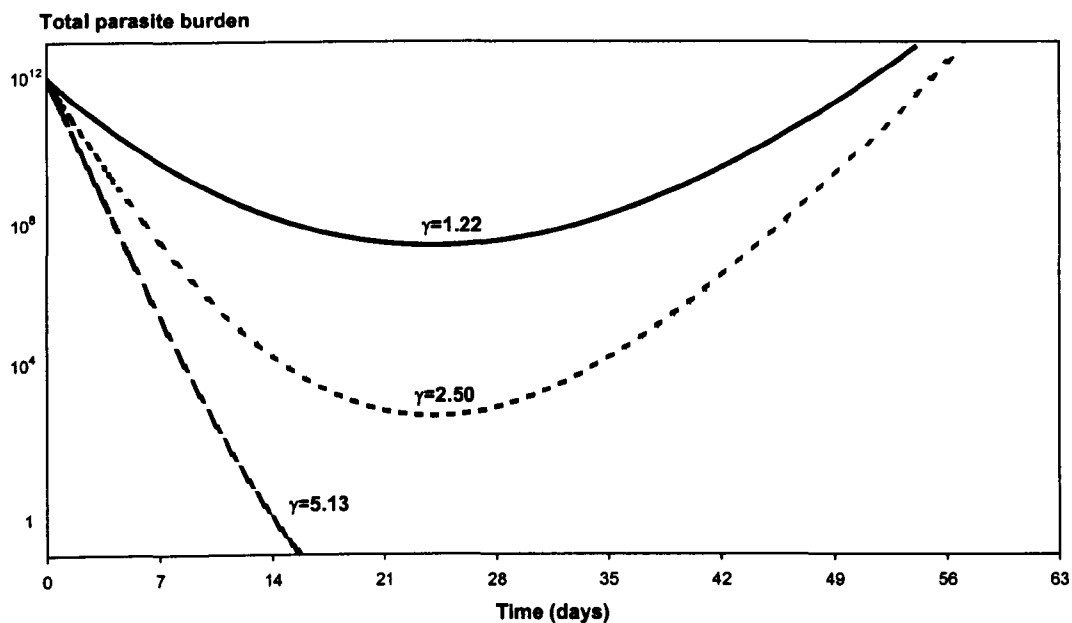
$P_0$  – total parasite burden at time zero;  $a$  – growth rate constant of the parasite;  
 $k$  – terminal elimination rate constant of mefloquine;  
 $k_1$  – rate constant of parasite killing;  $C_0$  – maximum concentration of mefloquine;  
 $\gamma$  - slope of the concentration effect curve;  
 $C_{50}$  – drug concentration which kills 50% of the parasites.

Other factors beside the dosage can affect the maximum concentration achieved. Acute malaria may affect absorption and the apparent volume of distribution of drugs because of reduced food intake, vomiting, reduced gastric motility and visceral blood flow. Figure 4.4 depicts the parasite-time profiles for the population mean  $C_{\max}$  following 25 mg/kg of mefloquine (defined  $C_0$  in the model) and for the minimum and maximum  $C_{\max}$  values of the 1000 simulated PK profiles. With a dose of 25 mg/kg the mean  $C_{\max}$  was 1200 ng/ml, with a range from 400 ng/ml to 7440 ng/ml. With the starting parameters of the model the absolute minimum  $C_{\max}$  required to cure an adult patient with an admission parasitaemia of 2% is 1400 ng/ml. Non-linear mixed effects modelling of the 1000 simulated PK profiles produced similar parameter and variance estimates to those used in the original simulation of the 1000 profiles (see Table 4.1).

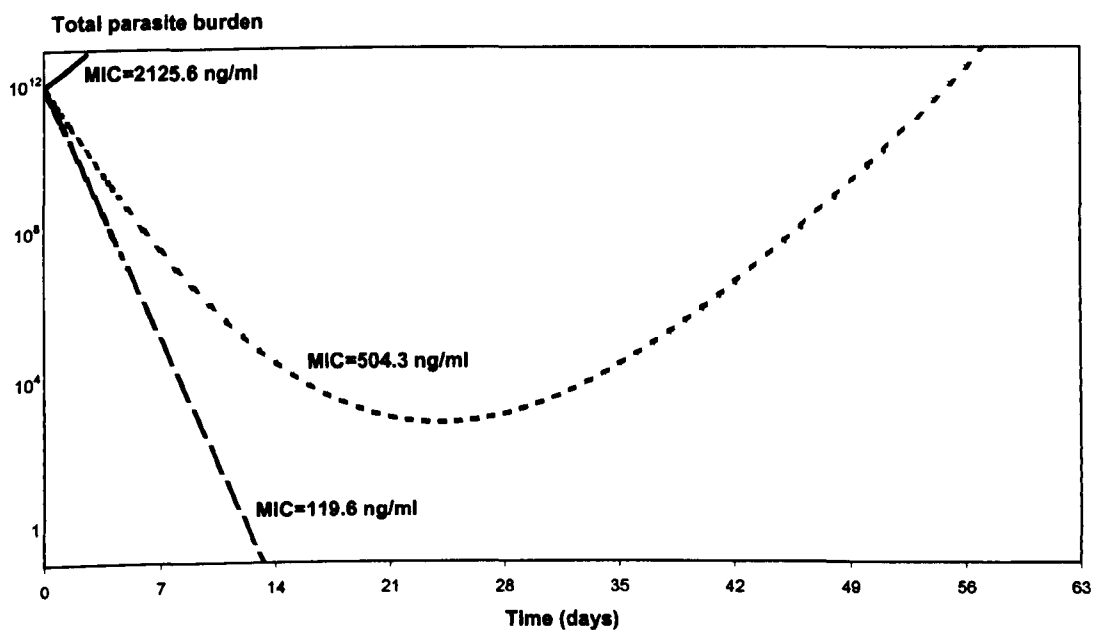


**Figure 4.4** Relationship between parasite clearance over time and maximum mefloquine concentration ( $C_0$ ). In this example,  $P_0$  is  $10^{12}$ ,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $k_1$  is 3.45 /day,  $\gamma$  is 2.5, and  $C_{50}$  is 665.4 ng/ml.

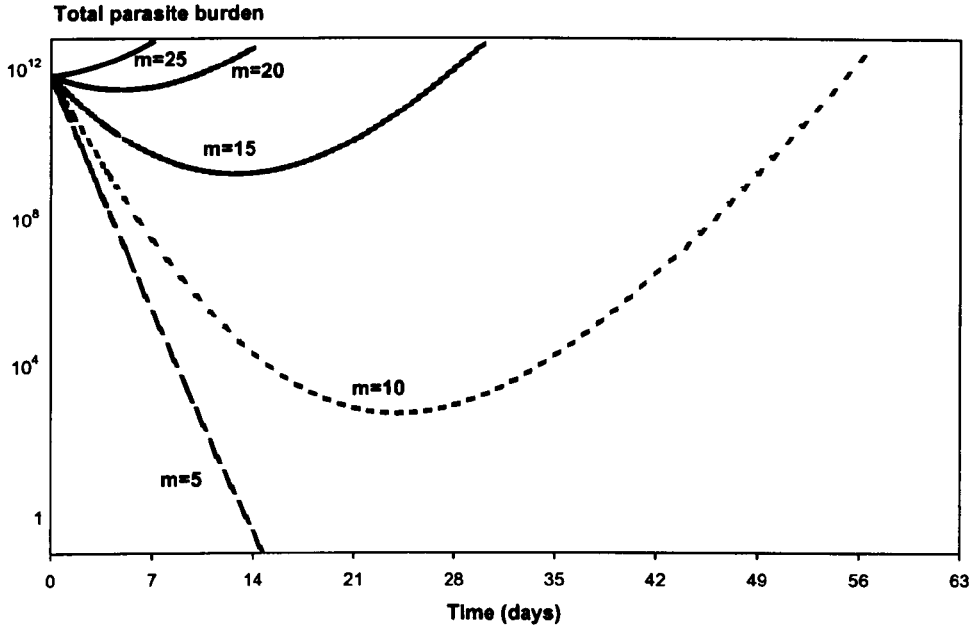
The effects of varying values of the parameters  $\gamma$ ,  $EC_{90}$  and  $m$  on the parasite versus time profiles are shown in Figures 4.5, 4.6 and 4.7. The flatter the slope of the concentration-effect curve the more likely the patient will suffer a subsequent recrudescence of the infection (Figure 4.5). For the lower limit ( $\gamma=1.22$ ) and the population mean estimate ( $\gamma=2.50$ ) the parasites never cleared and started to rise again around 3 weeks following treatment. Patients will start to fail after treatment for mefloquine if the slope of the concentration-effect curve drops below 3.5. Increases in the *in vivo* MIC will also increase the risk of treatment failure (Figure 4.6). For example if the *in vivo* MIC is 120 ng/ml, all the parasites are cleared from the body in less than 2 weeks. If the MIC *in vivo* increases to 2126 ng/ml (the upper limit of the 95% prediction interval of the population estimate), then the parasitaemia will continue to rise following treatment ( $R_3$  resistance). If the MIC *in vivo* is 5 fold the  $EC_{90}$  *in vitro* (i.e.  $m=5$ ) then all the parasites are cleared from the body at 2 weeks (Figure 4.7). However if the MIC *in vivo* is at least 20 times larger than the  $EC_{90}$  *in vitro* (i.e.  $m=20$ ), the parasitaemia keeps rising following treatment. For  $m=10$  and  $m=15$  the parasites never clear and start to rise again at around 3 weeks and 2 weeks respectively.



**Figure 4.5** Relationship between parasite clearance over time and the slope of the concentration-effect curve ( $\gamma$ ). In this example,  $P_0$  is  $10^{12}$ ,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $C_0$  is 1200 ng/ml,  $k_1$  is 3.45 /day, and  $C_{50}$  is 665.4 ng/ml.



**Figure 4.6** Relationship between parasite clearance over time and the MIC *in vivo*. In this example,  $P_0$  is  $10^{12}$ ,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $C_0$  is 1200 ng/ml,  $k_1$  is 3.45 /day, and  $\gamma$  is 2.5.



**Figure 4.7** Relationship between parasite clearance over time and  $m$  (scalar value relating  $EC_{90}$  *in vitro* to MIC *in vivo*). In this example,  $P_0$  is  $10^{12}$ ,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $C_0$  is 1200 ng/ml,  $k_1$  is 3.45 /day,  $\gamma$  is 2.5 and  $EC_{90}$  *in vitro* is 50.43 ng/ml.

#### 4.3.4 Ratio of $C_{max}$ to MIC *in vivo*

The ratio of the maximum plasma concentration to the MIC, a widely used parameter in anti-infective drug PD modelling, is probably an important determinant of outcome in the case of antimalarial treatment with mefloquine.

The function  $P_t$  can be rewritten as:-

$$P_t = P_0 * e^{a.t} * \left[ \frac{\left( \frac{(k_1 - a)}{a} \right) R^{-\gamma} + (e^{-k.t})^\gamma}{\left( \frac{(k_1 - a)}{a} \right) R^{-\gamma} + 1} \right]^{k_1/(k.\gamma)}$$

$$\text{where } R = \frac{C_{max}}{MIC}$$

$R$  will obviously decrease with increasing resistance in areas where the treatment dose is fixed, and  $R$  will remain constant if the treatment is increased commensurate with the increase in resistance.

Figure 4.8 shows the parasite-time profiles for  $R=1, 1.5, 2$  and  $3$ . If the  $C_{\max}$  is three times greater than the MIC the patient will be cured of malaria (all the asexual parasites will be cleared from the body within three weeks). The infection will recrudescence if the  $C_{\max}$  achieved is only 2 fold the *in vivo* MIC.

We can define  $R_C$  as the minimum value of  $R$  where the patient will still be cured of malaria.  $R_C$  occurs when the minimum value of  $P_t$  is less than or equal to 1. To find the minimum value of  $P_t$  the derivative  $\frac{dP}{dt}$  is set equal to zero and solved for  $t$ . This gives the time when the minimum total parasite burden occurs:-

$$t_{\min} = \log_e(R^{1/k})$$

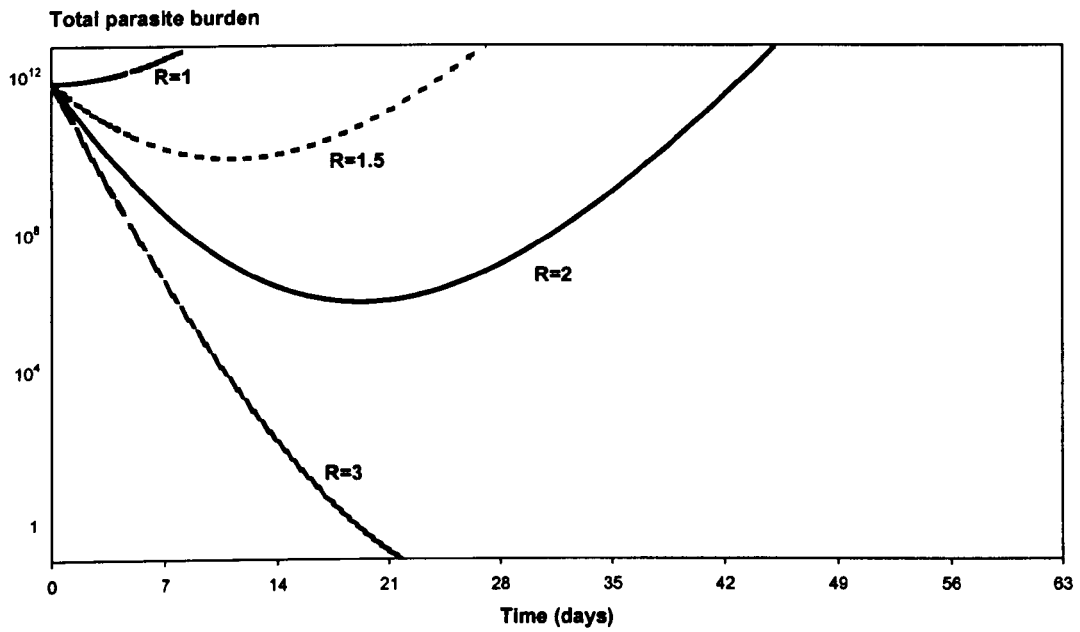
Substituting  $t_{\min}$  into  $P_t$  gives the following equation:-

$$P_0 * R^{a/k} * \left[ \frac{\left( \frac{k_1 - a}{a} \right) + 1}{\left( \frac{k_1 - a}{a} \right) + R^r} \right]^{k1/(k.r)} \leq 1$$

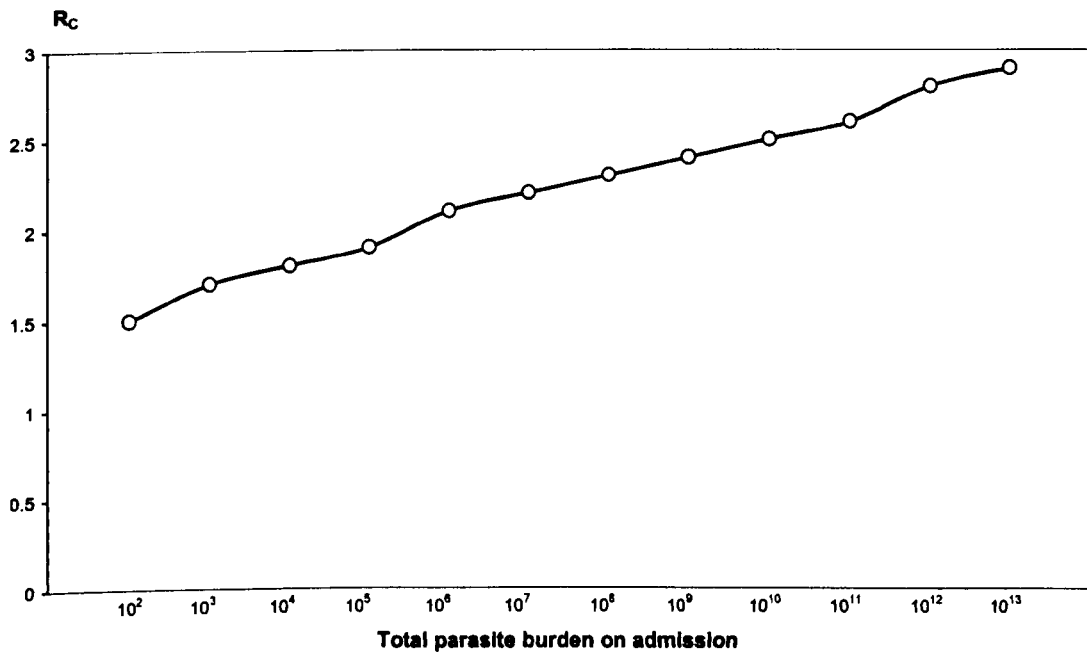
The above equation cannot be solved analytically, therefore  $R_C$  is derived numerically.

Figure 4.9 plots  $R_C$  over the full range of possible parasite burdens.





**Figure 4.8** Relationship between parasite clearance over time and the ratio of maximum mefloquine concentration ( $C_0$ ) to MIC ( $R$ ). In this example,  $P_0$  is  $10^{12}$ ,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $k_1$  is 3.45 /day, and  $\gamma$  is 2.5.



**Figure 4.9** Relationship between the minimum value of  $C_0$  to MIC ratio ( $R_c$ ) required to clear all parasites and the total parasite burden on admission. In this example,  $a$  is 1.15 /day,  $k$  is 0.036 /day,  $k_1$  is 3.45 /day, and  $\gamma$  is 2.5.

#### 4.3.5 Benefit of M25 over M15

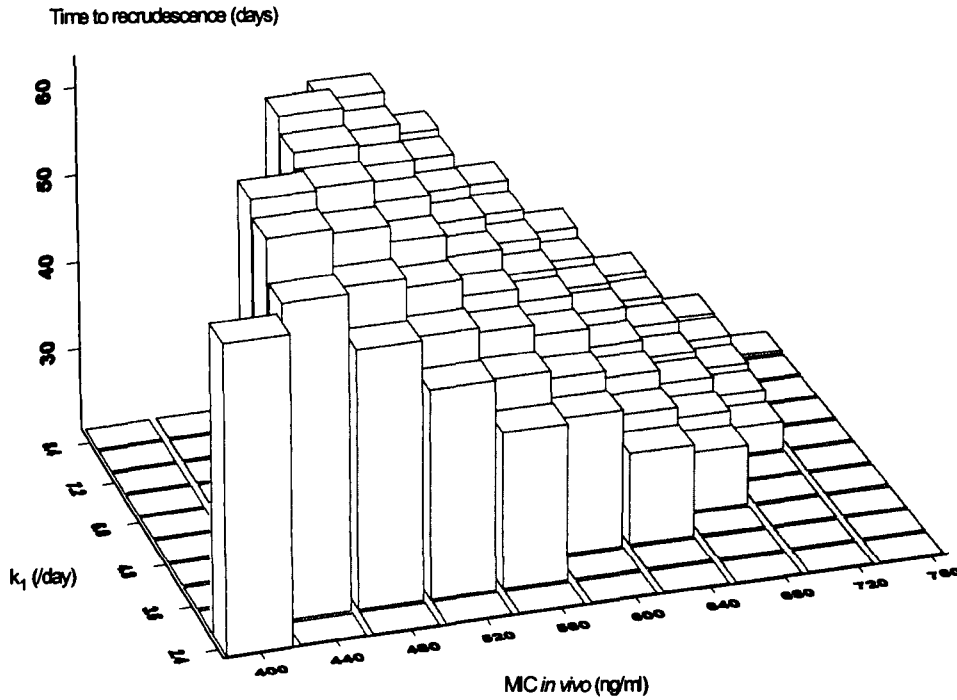
As stated earlier, Figure 4.3 shows clearly that at all times following drug administration to comparable infections the total parasite burden is greater for those patients receiving 15 mg/kg compared with the larger dose of 25 mg/kg.

If we assume that when the whole blood mefloquine concentration falls below 600 ng/ml the patient is exposed to drug levels which give submaximal effects (i.e. 600 ng/ml represents the minimal parasitocidal concentration (MPC)) then blood mefloquine levels fall below the MPC on day 6 for those patients receiving 15 mg/kg, and on day 20 for those receiving 25 mg/kg. On these days the total parasite burden predicted for patients receiving 15 mg/kg of mefloquine in the earlier example was  $4.87 \times 10^{10}$  parasites and for those receiving 25 mg/kg was only 1152 parasites, this equates to a  $4.23 \times 10^7$  fold difference.

#### 4.3.6 Time to recrudescence

The WHO 28 day parasitological classification (World Health Organisation, 1973) has four classifications for therapeutic response; sensitive, RI resistance, RII resistance and RIII resistance. Patients classified as sensitive have cleared their asexual parasites by day 7 without subsequent recrudescence between days 8 and 28. For RI (low grade) resistance the asexual parasites have cleared by day 7 but have reappeared by day 28. The day the parasites reappear microscopically is termed the time of recrudescence. For RII (intermediate grade) resistance the asexual total parasite count has reduced by at least 75% at 48 hours but has not cleared by day 7. For RIII (high grade) resistance the asexual total parasite burden either remains static or increases or reduces by  $< 75\%$  of the admission parasite burden at 48 hours.

To determine the clinical efficacy of the drug it is important that patients are followed up long enough so that a patient that has actually failed (RI resistance) has not been classified as cured. The interval between administration of antimalarial treatment with mefloquine and recrudescence of the patient's infection depends on the killing rate of the drug and resistance of the parasites. Figure 4.10 is a 3 dimensional plot illustrating the relationship between three parameters; the time of recrudescence,  $k_1$ , and the *in vivo* MIC. To create Figure 4.10 parasite versus time curves were simulated for patients receiving high dose mefloquine 25 mg/kg. The PK parameters used were the mean parameter estimates from the population PK modelling of mefloquine monotherapy;  $C_{max}=1200$  ng/ml and  $k=0.036$  /day (Chapter 3). Those curves where the parasite burden fell below the level of detection (defined as 50 parasites / $\mu$ l or  $2.5 \times 10^8$  parasites) before day 7 were selected. If  $k$  was  $\leq 1.2$  /day and the MIC  $\geq 360$  ng/ml the parasitaemia does not clear by day 7 (RII or RIII resistance). If the MIC was  $< 360$  ng/ml and  $k > 1.2$  /day the patient was cured (drug sensitive parasites). The time of recrudescence is the time at which the parasites can be detected again microscopically after falling below microscopic levels in the first week following treatment (defined as RI resistance). As  $k_1$  increases, that is the drug has a higher PRR or greater parasitocidal activity, cure rates will increase, but in those few failures which do occur, the interval from treatment to recrudescence occurs later. With higher levels of resistance (i.e. a high MIC) patients' infections will recrudescence earlier. The time to recrudescence was normally distributed with a mean (SD) of 42 (11) days ranging from 20 up to 64 days. Table 4.4 presents the distribution of times to recrudescence for different levels of resistance (i.e. varying MICs *in vivo*).



**Figure 4.10** Time to recrudescence following treatment with mefloquine at 25 mg/kg as a function of MIC *in vivo* and killing rates of the drug mefloquine ( $k_1$ ). The *z*-axis is the time to recrudescence (days), the *y*-axis is the killing rate ( $k_1$ ) of mefloquine (/day), and the *x*-axis is the MIC *in vivo* (ng/ml).

The pharmacokinetic parameters used in the simulation were  $C_0 = 1200$  ng/ml and  $k = 0.036$  /day.

Nonraised rectangles represent two possible scenarios: the patient is cured or at day 7 the parasites are still detectable. This illustrates that for relatively drug-sensitive parasites (MIC < 500 ng/ml) the infections are all cured with high killing rates and that with low killing rates recrudescences occur long after the conventional follow-up period of 28 days. Conversely, with highly resistant parasites, long follow-up (i.e., > 42 days) is not necessary.

**Table 4.4:- Distribution of time to recrudescence for varying levels of resistance.**

MIC <i>in vivo</i> (ng/ml)	Time to recrudescence (days)	
	Mean (SD)	Range
500	56.0 (6.2)	45 – 64
525	52.0 (5.5)	42 – 59
550	47.4 (4.9)	38 – 53
575	44.0 (3.8)	37 – 49
600	40.0 (3.2)	34 – 44
625	36.4 (2.8)	31 – 40
650	33.0 (2.3)	29 – 36
675	30.0 (1.7)	27 – 32
700	26.8 (1.3)	25 – 28

MIC – minimum inhibitory concentration; SD – standard deviation.

#### 4.4 Discussion

In this chapter a mechanistic model describing the change of total parasite burden over time (in the presence of drug) *in vivo* was derived. The model was dependent on seven different parameters; parasite burden before treatment, parasite multiplication rate, PK parameters (maximum concentration and terminal elimination rate constant of the drug) and PD parameters (rate constant of parasite killing, slope of the concentration-effect curve and drug concentration which kills 50% of the parasites).

The model was developed for the treatment of malaria with mefloquine monotherapy. Parasite burden over time profiles were predicted for different dosages of mefloquine, for different levels of disease severity (defined by admission parasite burden), and for predicting the time when a patient will recrudescence for differing levels of mefloquine resistance. The model enables a clinician to investigate the *in vivo* consequences of

changes in a drugs PK profile, predict time to recrudescence, and predict relative risks for selecting resistant parasites (e.g. exposure to subtherapeutic drug concentrations).

#### 4.4.1 Different dosages of mefloquine

The development of resistance to antimalarial drugs is a major threat to the health of people living in tropical countries. The speed at which resistance develops is affected considerably by the pattern of antimalarial drug use. Uncontrolled self medication with inadequate treatment courses provides a particularly potent selection pressure to the development of resistance as it exposes a large number of parasites to partially effective antimalarial drug concentrations. In this chapter the effects of two different systematic antimalarial treatment strategies (mefloquine monotherapy:- 25 mg/kg and 15 mg/kg) were examined. This is a particularly relevant example as resistance to mefloquine has developed rapidly on the Eastern and Western borders of Thailand, and the adjacent areas, and is now emerging separately in Vietnam. This could have been delayed or perhaps prevented.

When new drugs are introduced, dose ranging studies are performed to determine the optimum dose and treatment schedule based on the therapeutic ratio (maximum efficacy / minimum toxicity). For most anti-infective drugs, with the notable exception of antituberculous and antiretroviral drugs, the prevention of resistance is usually not considered in this assessment. This conventional therapeutic appraisal usually leads to the conclusion that the smallest dose giving an “acceptable” cure rate should be deployed initially. For slowly eliminated antimalarial drugs, such as mefloquine, this will lead more rapidly to resistance than if a higher dose were deployed initially. There are two reasons for this. Firstly, the lower dose chosen initially, which for mefloquine was 15 mg base/kg, provides greater opportunity for the *de-novo* selection of genetic mutants, such as those containing increased copy numbers of the *pfmdr1* gene, with significantly reduced

susceptibility (Price et al. 1999b). This is because there is a greater probability of failing to achieve concentrations that would kill a mutant parasite and its progeny. Secondly, once these mutants have arisen and have been transmitted, then, provided they are highly mefloquine resistant, they will be more likely to survive low dose treatment in subsequent hosts. This increased survival probability results in an increase in the proportion of resistant mutant parasites. Thus resistance is selected more efficiently by the general use of a lower (15 mg/kg) than higher (25 mg/kg) dose. It should be noted that the simulated parasite burden-time profiles represent an area of low transmission where background immunity is low or absent (such as much of South East Asia and South America). In areas of high stable transmission host immunity may be sufficient to clear resistant infections. In addition a significant proportion of infections are transmitted from individuals who do not receive antimalarials. These two factors reduce the selection of resistant mutants and retard the evolution of drug resistance.

Cost is a very important factor determining antimalarial drug use. If an antimalarial drug treatment costs 60% more (as would be the case for mefloquine high dose versus low dose), this could impose a significant financial burden on developing countries. However this must be balanced by the costs of resistance, both in terms of morbidity, and perhaps mortality, and also the need to fund newer and usually more expensive alternative drugs. The data and modelling predictions presented here provide a framework for such a pharmacoeconomic assessment.

#### 4.4.2 Disease Severity

Patient cure and the development of drug resistance are both dependent on the initial total parasite burden of the patient (ter Kuile et al. 1995b). For a patient to be radically cured (i.e. all of the parasites in the body are eradicated) an effective antimalarial drug concentration needs to be present in the blood until either the last parasite has been

removed or immune defences are able to kill the remaining few parasites. Patients with a high parasite burden on admission will take longer to clear all their parasites because they have more parasites to remove. For drug resistance, if one malaria parasite in every  $10^x$  malaria parasites contains a mutation that confers a significant reduction in susceptibility to the drug being used then the probability of the patient harbouring such a resistant mutant is equal to  $\frac{1}{10^x} \times P_t$  (where  $P_t$  is the number of parasites in a single patient at time  $t$ ). Thus if at time zero there are more parasites there is a greater probability that a resistant mutant will emerge. Increasing the dose of mefloquine ensures that concentrations remain above the minimum parasitocidal concentration longer and therefore gives a greater chance of eradicating all of the parasites from a hyperparasitaemic infection.

#### 4.4.3 Time to recrudescence

Antimalarial drug resistance is commonly underestimated. When *in vivo* drug assessments are performed patients are followed usually for between one and four weeks. This is insufficient for mefloquine. The model presented here explains that recrudescences may occur well after one month following treatment. Furthermore early in the evolution of resistance (i.e. a low MIC), when there are few recrudescences, a greater proportion of recrudescences will occur after 28 days. Thus the degree to which mefloquine resistance is underestimated (failures  $\leq 28$  days divided by total failure rate) is greater at low levels of drug resistance. As resistance worsens the mean time to recrudescence shortens. The simulations presented in Figure 4.10 provide estimates of the time to recrudescence for areas with different levels of mefloquine resistance.



#### 4.4.4 Limitations of the model

The parasitological response to an antimalarial drug is influenced by many factors. These are the parasites' drug susceptibility, host factors (e.g. natural immunity), PK factors (e.g. drug absorption), PD properties of the antimalarial drug (e.g. rate constant of parasite killing), and the intrinsic characteristics of the infection (e.g. parasite burden, and stage and synchronicity of parasite development at the time of drug administration). The model presented in this chapter includes parameters to represent the PK and PD properties of mefloquine, the parasites' susceptibility to mefloquine, and the initial parasite burden of the infection. However there are no parameters to represent the hosts' background immunity to malaria, and the initial stage and synchronicity of parasite development.

Excluding a parameter to represent the level of the host's background immunity to malaria is probably acceptable for areas with low transmission and little host immunity, such as South East Asia. However for areas with high transmission, such as Africa, the model will predict a "worse scenario" in terms of parasite clearance compared with that actually observed. To include a function for background immunity is difficult since there is no direct measure of immunity. One possible solution to this problem is to change the parasite multiplication rate ('a') to a value called the 'adjusted parasite multiplication rate'. The 'adjusted parasite multiplication rate' could be set at different values for immune, semi-immune and partly immune patients. Again however it is not possible to estimate from data the 'adjusted parasite multiplication rate' for patients with different levels of natural immunity. Current estimates for the 'parasite multiplication rate' in the absence of drug are derived from patients with very little or no background immunity to malaria (Molineaux and Dietz, 1999).

The stage and synchronicity of the *P. falciparum* infection can complicate the characterisation of the PK-PD relationship. *P. falciparum* has a 48 hour life cycle, during

which the parasite develops from tiny young ring forms, to pigmented trophozoites and then mature segmenting schizonts. Mefloquine is most active *in vitro* against the mature trophozoite and schizont forms (Geary et al. 1989). If a synchronous infection is treated at a time when the majority of the parasites are at the late schizont or tiny ring stages, the parasitaemia may rise after treatment as new rings are formed following merogony. The model presented in this chapter does not account for the stage-dependency of mefloquine and the synchronicity of the infection. Instead the model will predict for infections with varying distributions for the age of their parasites exactly the same parasite versus time profiles conditional that all other parameters are the same. For mefloquine, a slow-acting drug, the stage-dependence is probably less important because this dependence is smoothed by the long period during which mefloquine remains active in the body. However for artemisinin (a fast acting drug) it might be more important to relate the antiparasitic effect to stage-specificity and parasite synchronicity than to the PK for predicting clinical efficacy and determining dosage intervals (White, 1997; Hoshen et al. 2000b).

#### 4.4.5 Limitations of the parameter estimates

The PD parameters, slope of the concentration-effect curve and the concentration which kills 50% of parasites, are unknown *in vivo*. The parasite-time profiles simulated were based on extrapolating the PD parameters derived from *in vitro* data. Although careful analysis was performed to obtain the estimates of the slope and  $EC_{90}$  *in vitro*, two major assumptions were made to extrapolate these values to give estimates of the slope and  $EC_{90}$  *in vivo*. Firstly it was assumed that the slope of the concentration-effect curve *in vivo* was the same as that observed *in vitro*. This assumption is very difficult to prove (or disprove). A different slope would imply a qualitatively different parasite transport system or enzyme kinetics for the supposed drug target *in vivo* and *in vitro*. In most other respects the malaria parasites behave the same *in vivo* and *in vitro*. In particular growth rates are comparable

and the population dynamics are similar. Secondly it was assumed that the  $EC_{90}$  *in vivo* was ten times larger than the  $EC_{90}$  *in vitro*. This number was chosen empirically and it can be seen in Figure 4.7 that changing the value from 5 up to 20 can have huge implications in terms of predicting patient cure. However it is difficult to determine what inflation factor should be applied to the  $EC_{90}$  *in vitro*. Plasma or whole blood drug levels measured *in vivo* include free (unbound) drug and protein bound drug. Only the drug that is not bound in plasma is capable of crossing membranes and killing the malaria parasites. Thus *in vivo* the amount of free concentration that kills the parasite is much lower than the actual concentration measured due to plasma protein binding. In other words the *in vivo* concentration-effect curve is shifted to the right of the *in vitro* concentration-effect curve by an unknown amount. This shift in the curve results from protein binding. *In vitro* attempts to assess mefloquine plasma protein binding have been confounded by the tendency of mefloquine to stick to plastics and other laboratory ware. Protein binding is generally considered to be very high, approximately 98% (Karbwang and White, 1990).

The *in vitro* parameters were derived from modelling of *in vitro* concentration-effect curves where the effect measure was inhibition of parasite uptake of [ $^3H$ ] hypoxanthine. The inhibition of hypoxanthine uptake, is a measure that correlates with growth inhibition of parasite multiplication but it does not equate with it.

## **5. POPULATION DYNAMICS OF THE *PLASMODIUM FALCIPARUM* PARASITE WITHIN THE HUMAN HOST IN THE ABSENCE OF ANTIMALARIAL DRUGS.**

### **5.1 Introduction**

The discovery in 1917, by the Viennese psychiatrist Professor Julius Wagner Ritter von Jauregg, that a significant proportion of patients with neurosyphilis would improve following an attack of malaria led to a novel form of treatment for, what was then, an essentially incurable disease (Wagner-Jauregg, 1922). At that time approximately 10% of all inpatients in mental institutions in Britain had neurosyphilis. A four year cumulative mortality of 80% fell to less than 10% following the introduction of malaria therapy. Patients with neurosyphilis were treated by inducing a protracted attack of falciparum or vivax malaria. Both mosquito borne and blood borne infections were induced. Laboratories were established in mental hospitals in Europe and the United States for such treatment (Collins and Jeffery, 1999). Relatively few isolates of the malaria parasites were used in the several major laboratories using this method. For example the Madagascar "strain" of *Plasmodium vivax* was used to infect over 10,000 patients during a 30 year period at the Horton Hospital in England. In most centres using malaria therapy, detailed records were routinely kept of symptoms, fever, parasite counts and the responses to treatment, in primary infections or in reinfections with both heterologous and homologous strains or species of parasites. These extensive records provide a unique source of information about the immunology and natural history of malaria in both the non-immune and previously infected adult human hosts. In addition, considerable experience was acquired in the modulation and elimination of infection, usually with chemotherapeutic agents. There was

no evidence that the presence of syphilis or the chemotherapy used for syphilis modified significantly the progression of the malaria infection (Winckel, 1941).

Following the introduction of sporozoites, either by mosquito bite or injection, there is a phase of (pre-erythrocytic) development, which lasts approximately one week in hepatocytes (hepatic schizogony) before liberation of usually between ten thousand and a million motile parasites (merozoites) into the blood. These parasites then invade red cells and begin repetitive cycles of development and asexual reproduction in red cells. This leads to red cell destruction (haemolysis) and when the density of parasites in the blood is significant, the illness malaria. *Plasmodium falciparum*, *P. vivax* and *P. ovale* have an approximate two day intra-erythrocytic life span following invasion of a red cell. At the end of the parasite's life cycle the mature *P. falciparum*-schizont bursts releasing up to 32 merozoites into the blood. The liberated merozoites invade red cells immediately. The microscopist can detect parasites only when the patient's parasitaemia exceeds 10 parasites / $\mu$ l. This corresponds to greater than 100,000,000 parasites in the blood of an adult patient (White, 1998). This also corresponds approximately to the threshold for symptoms of illness. Thus after hepatic schizogony the first few asexual lifecycles are not detectable microscopically and the patient is well. Parasitaemias are detected, on average, 10-13 days following inoculation (Jeffery et al. 1959).

A pictorial representation of *P. falciparum* parasitaemia over time from a patient with an induced infection is an oscillatory profile around an exponential rise in parasitaemia in the first week followed by a plateau and an eventual decline. The oscillation is present because approximately half way through the asexual cycle the *P. falciparum*-infected erythrocytes adhere to the capillary and venular walls (a process known as sequestration) and can no longer be seen microscopically (White et al. 1992a).

The usual progression of malaria, even in a naïve host, is for the exponential increase in parasite numbers to stop (relatively abruptly). An equilibrium then occurs for days or weeks before parasite numbers begin to decline. After the acute illness low levels of parasites remain in the blood for several weeks or months before finally being eliminated. Only rarely does the multiplication of *P. falciparum* continue unchecked to result in life threatening parasite burdens. There are several reasons for this. One possible reason is the competition between parasites for host erythrocytes. In the past it has been assumed that *P. falciparum* invasion of erythrocytes is a random process. However, Simpson et al. (1999) found that the process is not random in the majority of patients. On average only 40% of circulating red cells were available for invasion for patients' with *P. falciparum* parasitaemia < 2%. Non-selective invasion was found in patients with severe malaria (Simpson et al. 1999) and was later shown to be a parasite property, and probable virulence determinant (Chotivanich et al. 2000a). Other factors that limit parasite multiplication are non-specific and eventually the development of specific immune defences from the human host. Schizonts reducing their multiplication potential may also be damaged by high fevers (Kwiatkowski, 1989; Kwiatkowski et al. 1990).

The aim of this chapter was to estimate the parasite multiplication rate (PMR) and periodicity of the parasite cycle in the first week of patent parasitaemia in the absence of antimalarial treatment. Both PMR and periodicity of the parasite cycle are important parameters of mathematical models that predict the pharmacodynamics *in vivo* of antimalarial agents (White, 1997); the within-host dynamics of *P. falciparum* malaria (Molineaux and Dietz, 1999; Hoshen et al. 2000a); and the transition of asexual parasites to gametocytes (Diebner et al. 2000). Following antimalarial drug treatment the parasitaemia may fall as a result of sequestration or rise as a result of schizogony and merozoite release. These may erroneously be considered to represent success or failure respectively of treatment, yet they are the natural consequences of the infection. Such

changes need to be characterised before drug effects can be assessed. The period of initial growth represents the time when host defences have their least effects on the parasite population. The estimates of the two parameters, PMR and periodicity, were compared between different *P. falciparum* strains; and sporozoite and blood induced infections.

## **5.2 Methods**

### **5.2.1 Patient data**

The basis for the present retrospective analysis consists of data extracted from parasitologic and fever records acquired between 1940 and 1963 at National Institutes of Health installations then located at the South Carolina State Hospital in Columbia, South Carolina and the Georgia State Hospital in Milledgeville, Georgia (Collins and Jeffery, 1999), where malaria therapy was being routinely used in the treatment of neurosyphilis. An ethical analysis of the retrospective usage of these data for research revealed that there was no evidence that the patients were exploited (Weijer, 1999). The raw data were compiled by William E Collins and Geoffrey M Jeffery, who also provided the raw data and the data base to the Department of Medical Biometry, University of Tübingen, where the data have been computerised.

Three species of *Plasmodium* (*P. falciparum*, *P. malariae* and *P. ovale*) were used in the malaria therapy of the African-American patients; *P. vivax* was ruled out because of genetic resistance to this species (Miller, 1976). *Plasmodium falciparum* was most frequently used because of the more desirable infection characteristics, availability, ease of transmission, and other factors; all of the infections considered in this analysis consisted of a number of strains of this species. Each patient was under close medical supervision. Consent for whatever treatments the hospital staff determined necessary was granted by the families or the courts when the patients were admitted to the hospitals.

Three hundred and twenty-eight of the patients infected with *P. falciparum* for the treatment of paresis and other mental disorders associated with tertiary syphilis were studied. All were primary infections. Patients were infected from one of the following 10 different *P. falciparum* strains, where 'n' represents the number of patients infected with the corresponding strain:-

- 1) McLendon [n=141]: The McLendon strain was isolated in October 1940 from a patient who was admitted to the South Carolina State Hospital with malaria (Young et al. 1943; Young et al. 1948).
- 2) El Limon [n=91]: The El Limon strain was isolated from a resident of El Limon, Transisthmian Highway, Panama in 1948 (Jeffery et al. 1950).
- 3) Santee Cooper [n=81]: The Santee Cooper strain was isolated during the summer of 1946 from a patient living in the vicinity of the Santee Cooper impoundments in South Carolina (Eyles and Young, 1950).

The seven other strains provided by various investigators were Colombia (n=6), Costa (n=3), Thailand (n=2), So.Rhode (n=1), Strain\_ "7" (n=1), V-40G (n=1) and Walker (n=1).

All patients remained in screened wards to prevent infection of local anophelines. Patients were infected either by the infection of parasitised blood or via sporozoite inoculation. With the latter, inoculation was either by the bites of infected anopheline mosquitoes or by the subcutaneous or intravenous inoculation of sporozoites dissected aseptically from mosquito salivary glands. For some patients treatment with non-curative doses of antimalarial drugs (primarily 325 or 650 mg of quinine sulphate) was given to modify and control the early stages of infection. Any history of recent malaria infection was also recorded. Thick and thin peripheral blood films were made daily, stained with Giemsa stain, and examined microscopically for the presence of parasites. Parasites were recorded



per  $\mu\text{l}$  of red blood cells as determined by the method of Earle and Perez (1932). The detection threshold was approximately 10 parasitised red blood cells per  $\mu\text{l}$ . Patients' temperature, pulse, and respiration were checked every four hours and hourly during episodes of fever by hospital personnel.

### 5.2.2 Mathematical model

To date no adequate mechanistic model has been found to describe the data from the neurosyphilis patients (Molineaux and Dietz, 1999). Therefore the mathematical model fitted to the data in this chapter was empirically derived.

During the initial phase of the malaria infection the number of parasitised erythrocytes rise exponentially, giving

$$\frac{dY}{dt} = bY$$

where

$Y$  – parasitaemia (/  $\mu\text{l}$ )

$t$  – time (days)

$b$  – growth rate constant of the parasite (/day)

This equates to:-

$$\log_{10} Y = a + (b \times t)$$

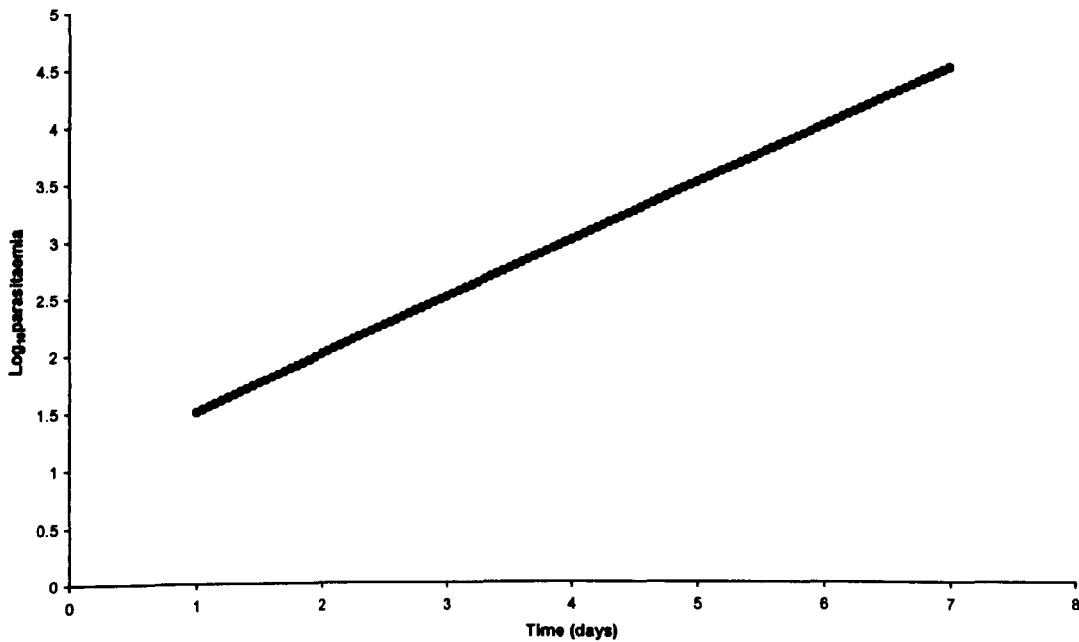
where

$a$  – parasitaemia ( $\log_{10}$  units) at time 0

The parasite multiplication rate (PMR) every two days is equal to  $10^{2b}$ . Thus the following equation was fitted to the data to obtain the parameter of interest directly.

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t) \quad \dots\dots(5.1)$$

For a PMR of 10 and a parasitaemia of 10 / $\mu$ l at time 0 (i.e.  $a=1$ ), equation 5.1 predicts the following parasitaemia versus time curve for the first 7 days of patent parasitaemia (Figure 5.1).



**Figure 5.1.** Predicted parasitaemia versus time profile for equation 5.1 where PMR=10 and  $a=1$ .

Unfortunately the parasitised cells that are seen microscopically do not represent all the parasites in the body. Erythrocytes containing mature parasites sequester in the microvasculature of vital organs (White and Ho, 1992b), and are therefore no longer microscopically visible. In other words on one day one may see parasitaemias reflecting

nearly all the parasites in the body, yet 24 hours later only a small proportion are visible (White et al. 1992a). Parasite sequestration causes the parasitaemia time profile to oscillate regularly about the simple loglinear relationship illustrated above (Figure 5.1), provided the parasite age distribution is unimodal and symmetrical.

Therefore the following equation was fitted to the data,

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t) + c \times \sin\left(\frac{2\pi}{\text{period}} t + k\right) \quad \dots\dots(5.2)$$

where

Y – parasitaemia (/μl)

t– time (days)

a – parasitaemia (log<sub>10</sub> units) minus c\*sin(k) at day 0

PMR – parasite multiplication rate every 2 days

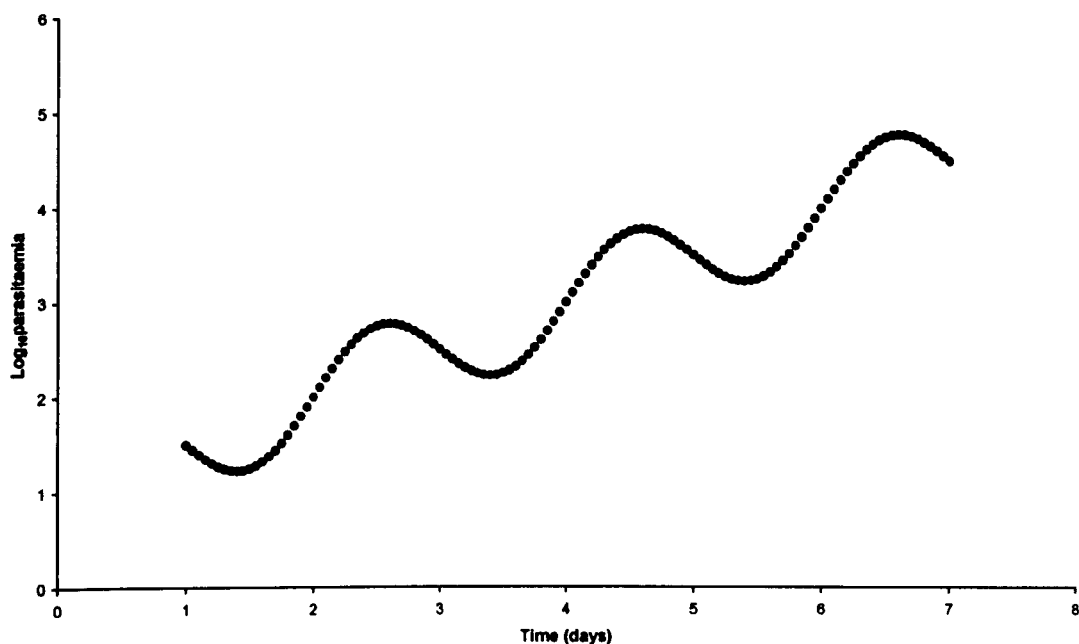
c – amplitude of sine wave

period – periodicity of sine wave

k – phase shift in sine wave

It was assumed that during the initial growth phase of the infection that parasite clearance was negligible. Data modelled by White et al. (1992a) indicated that there was little or no clearance of circulating rings in the first week of detectable parasites.

Setting the five parameters of equation 5.2 at the following values: PMR=10, a=1, c=0.5, period=2, k=0. Figure 5.2 represents the predicted parasitaemia versus time curve.



**Figure 5.2.** Predicted parasitaemia versus time profile for equation 5.2 where PMR=10,  $a=1$ ,  $c=0.5$ , period=2 and  $k=0$ .

Since the data are fitted empirically the following equations (listed below) were also tried.

All were less successful than equation 5.2.

$$\log_{10} y = a + (b \times t) + \frac{c \times \sin\left(\frac{2\pi}{\text{period}} t + k\right)}{t}$$

$$\log_{10} y = a * \exp(b \times t) + c \times \sin\left(\frac{2\pi}{\text{period}} t + k\right)$$

$$\log_{10} y = a * [1 - \exp(b \times t)] + c \times \sin\left(\frac{2\pi}{\text{period}} t + k\right)$$

The first seven days of microscopically visible parasitised erythrocytes were selected for statistical analysis. The first week of initial growth represents the time when host defences have little effect on the parasite population. If the age of the parasite is approximately 48

hours then over seven days 3 life cycles of the parasite will be observed. After seven days the majority of patients had parasitaemia-time profiles that had either reached a plateau or begun to decline.

### 5.2.3 Statistical Analysis

The data were analysed using three different statistical approaches; the standard two-stage method, the global two-stage method and nonlinear mixed effects modelling.

#### 5.2.3.1 Standard two-stage method (STS)

The first stage is to estimate the parameters 'a', 'PMR', 'c', 'period' and 'k' through nonlinear regression (least squares method) using an individual's dense parasite-time data. Individual parameter estimates obtained in the first stage serve as input data for the second stage calculation of descriptive summary statistics, typically mean (or log-mean) parameter estimates and variance of the individual parameter estimates. The variability around the mean population parameter is calculated only from the individual parameter estimates, no adjustment is made to take account of the uncertainty in the estimation of the individual specific parameters. This approach therefore overestimates systematically the inter-individual variability (Steimer et al. 1984). Each individual parameter is estimated from the original parasite-time data with some error. This error adds variability to the parameter estimates, thus increasing the estimate of the inter-individual variability. The STS method is only possible if there are sufficient data to estimate the model parameters for each individual, and if all data from each individual are described by the same structural model. The mathematical model in this paper has five parameters, and thus at least six data points per individual are required.

### 5.2.3.2 Global two-stage method (GTS)

The GTS method is the same as the STS method for the first stage. As well as the individual parameter estimates, their covariance matrix is obtained. At the second stage the GTS method refines the estimation of the inter-individual variability through bias correction and differential 'weighting' of individual data according to its quality and quantity. GTS estimates for the mean population parameters and their corresponding inter-individual variability are computed according to Prévost's recursive scheme (Prévost, 1977). The required assumption for the GTS method is that the distributions of the parameters and their corresponding covariance matrix are all multivariate normal (Steimer et al. 1984).

### 5.2.3.3 Nonlinear mixed effects modelling

The data for all individuals are analysed simultaneously, with mixed effects modelling taking into account both fixed and random effects. The fixed effects are the mean population parameters or a number of parameters that relate the parameters to other variables, such as strain, type of inducement, etc. The random effects are the inter-individual variability and intra-individual variability.

The nonlinear mixed effects modelling procedure (Lindstrom and Bates, 1990) of the SPLUS data programme (SPLUS 4.5 for Windows, Mathsoft, Inc., Cambridge, Massachusetts) was used to calculate estimates of the population parameters ('a', 'PMR', 'c', 'period' and 'k') and their respective inter-individual variances ( $\eta^a$ ,  $\eta^{\text{PMR}}$ ,  $\eta^c$ ,  $\eta^{\text{period}}$  and  $\eta^k$ ). The programme also provides estimates of the residual random intra-individual error ( $\epsilon$ ). Convergence was achieved when the objective value did not differ by more than some prespecified difference (e.g. 0.0001) and the programme returned the final estimates of the population parameters. The objective function (minus twice the log-likelihood of the data) was used to determine the model that best fitted the data. A significant drop in the

objective function from the general model to the restricted model was used to determine the final model. The change in the objective function was compared to a  $\chi^2$  statistic with degrees of freedom equal to the difference in number of parameters between the two models. The goodness of fit of each model was also determined by the precision of the parameter estimates and examination of the scatter plot of the residuals versus the predicted parasitaemia. The individual parameters were calculated with use of the posterior estimates.

Patient covariates (e.g. inoculation via sporozoites (IVS)), as a fixed effect, were incorporated into the model for PMR as shown below.

$$PMR_j = [PMR + \theta(IVS)] \times \exp(\eta_{j,PMR}),$$

where  $\theta$  is the coefficient of the categorical variable 'IVS' and represents the change in PMR resulting from malaria infection via sporozoite inoculation compared with inoculation of parasitised blood. A statistically significant improvement in the objective function, improvement in the precision of the parameter estimate (SE), and reduction in inter-individual and residual variability were used to determine the importance of the covariates as predictors.

#### 5.2.3.4 Assessment of the information in the experimental design

Parasite counts were recorded daily for all patients. To investigate the oscillating nature of the data using nonlinear mixed effects modelling the optimal experimental design would have parasite counts recorded at more frequent time points following the first observed parasite count. The accuracy of the parameter estimates produced from mixed effects modelling depends upon the number and timing of the samples (i.e. experimental design). Methods based on the Fisher information matrix have been developed and extended to population studies to evaluate experimental designs (Retout et al. 2001). The population Fisher information matrix was determined from the experimental design conditional on the

model and population parameter values. The standard error of each parameter was computed from the square root of the diagonal elements of the inverse of the Fisher information matrix.

### **5.3 Results**

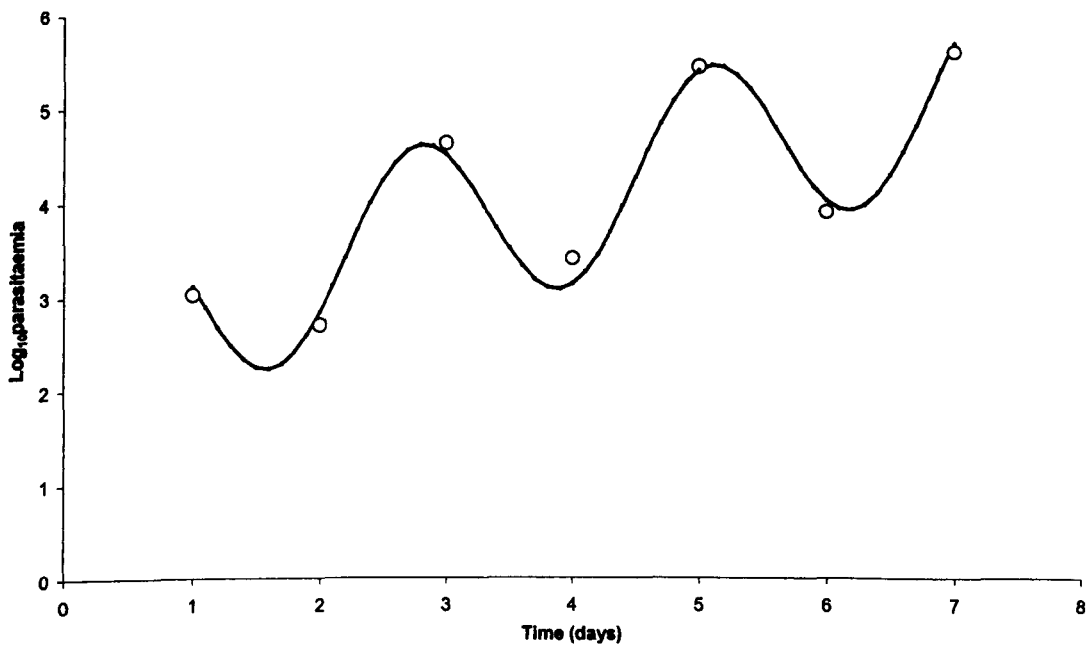
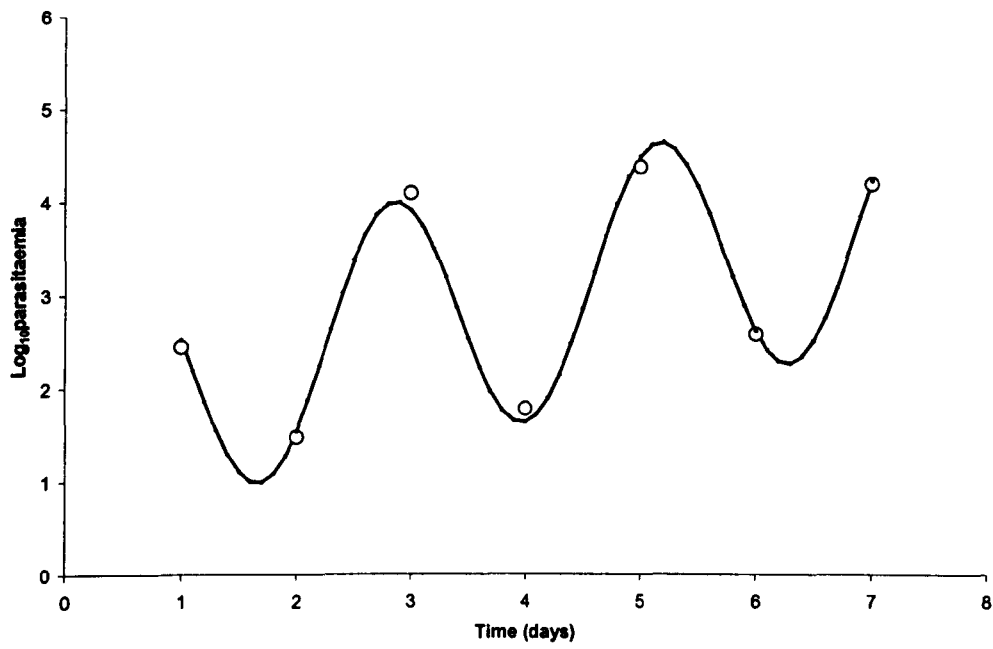
Eighty-three of the patients were excluded from the overall analyses because they had received antimalarial treatment during the first seven days of the infection. The implications of excluding these patients are addressed later in this section.

The remaining 245 patients had parasitaemia versus time modelled initially for each patient separately. By definition these patients had less severe infections than those who required rescue treatment. The model fitted the data adequately for 117 of the patients. The observed and fitted values for two of the patients are shown in Figures 5.3a and 5.3b. For 35 of the patients the five parameter mathematical model could not fit the data because there were less than six data points (e.g. parasitaemia peaked early or there were missing data). Visual inspection of the data of the remaining patients suggested the following reasons for the poor fits:-

- no oscillation (60)
- variable amplitude of the oscillation (16)
- shape very different from structural model (17)

The patients' for which the model could not be fitted to their parasite count data were a mixture of infections and were similar to the 117 patients where the model adequately described the data (Table 5.1).





**Figures 5.3a & 5.3b.** Parasitaemia versus time profiles of two patients during the rising phase of the infection.  
 ○ – observed values; line – predicted values.

Table 5.1 gives the demographics of the 117 patients who were included in the statistical modelling. Table 5.2 presents the estimates of the population parameters and their respective inter-patient variability produced by the three analytical methods; STS, GTS and nonlinear mixed effects modelling. Figure 5.4 shows a plot of predicted parasitaemia, estimated from the STS, GTS and nonlinear mixed effects methods, and the observed parasitaemia against time. Appendix V contains the SPLUS programme used for the nonlinear mixed effects modelling.

**Table 5.1:- Characteristics of the infections of the patients**

	N=117 (good fit of the model)	N=35 (not enough data points)	N=93 (poor fit of the model)
<i>Strain</i>			
El Limon	29.9%	17.1%	16.0%
Mc Lendon	41.0%	48.6%	46.8%
Santee	25.6%	28.6%	30.9%
Other	3.4%	5.7%	6.4%
<i>Method of infection</i>			
Sporozoite	37.6%	40.0%	20.4%
Blood	62.4%	60.0%	79.6%
<i>Gametocyteless strain</i>			
Yes	6.0%	5.7%	8.6%
No	94.0%	94.3%	91.4%

**Table 5.2:- Comparison of methods for modelling of the 117 patients’ data, comprising a total of 782 recorded parasite counts.**

	Standard two-stage (STS)	Global two-stage (GTS)	Nonlinear mixed effects modelling
Parameter	Estimate	Estimate	Estimate(SE)
a	1.33	1.34	1.31 (0.06)
PMR	8.11	8.26	8.20 (0.37)
c	0.56	0.62	0.50 (0.04)
Period	2.20	2.17	2.36 (0.02)
k	0.30#	-0.14	0.38 (0.09)
$\omega_a$	0.78	0.66	0.43
$\omega_{PMR}^*$	0.61	0.21 <sup>z</sup>	0.14
$\omega_c^*$	0.48	0.44 <sup>z</sup>	0.48
$\omega_{period}$	0.38	0.34	-
$\omega_k$	-	2.34	-
Residual SD - $\sigma_e$			0.485

SD – standard deviation; SE – standard error;

# - median;

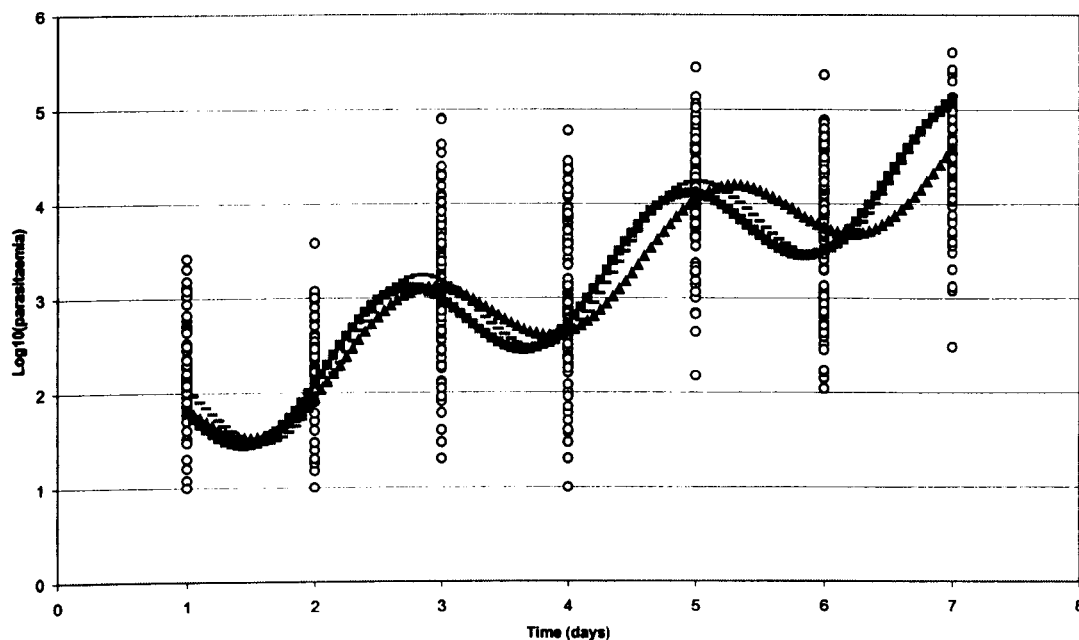
z – calculated using  $\text{var}(\log_e p) = (1/p^2) * \text{var}(p)$ ;

$\omega$  - inter-patient standard deviation for the parameters a, PMR, c, period and k;

\* - log-normal error model.

Mathematical equation fitted to data:-

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t) + c \times \sin\left(\frac{2\pi}{period} t + k\right)$$



**Figure 5.4.** Parasite counts (○) from 117 patients. Population predicted parasitaemia curves derived from STS (■), GTS (--) and nonlinear mixed effects modelling (▲).

The three methods STS, GTS and nonlinear mixed effects modelling gave similar population mean estimates for all of the parameters except 'k', the phase shift in the sine wave. The parameter 'k' is not normally or even lognormally distributed. This is why the estimates from STS and GTS differ so much, because the estimate obtained from the STS method is the median value. The estimates for the inter-patient variability of the parameters differed between the three methods with the STS method producing the highest estimates. It was only possible to produce an estimate of the residual (intra-patient) variation using nonlinear mixed effects modelling.

For nonlinear mixed effects modelling the inter-patient variability of the parameters 'PMR' and 'c' was assumed to be log-normally distributed. The parameter 'period' was observed to have negligible inter-patient variability in the mixed effects modelling and therefore the inter-patient variability of this parameter was not included in the final model. Surprisingly negligible inter-patient variability was observed for the parameter 'k'. Further investigation

showed that there was strong correlation between the inter-patient variability of parameters 'c' and 'k'. Including both in the model would result in overparameterisation. Thus only the inter-patient variability of parameter 'c' was included in the final model. An additive residual error (intra-patient error) model was selected to estimate the residual variability in the mixed effects modelling.

#### 5.3.1 Patients' with parasitaemia that peaked earlier than seven days or with missing data

Table 5.3 gives the results from nonlinear mixed effects modelling of 152 patients (including those 35 patients with missing data and early peaks). Nonlinear mixed effects modelling is capable of analysing sparse data as opposed to the other methods, STS and GTS. Comparing the estimates with those observed from the restricted dataset of 117 patients it can be seen that the estimates are similar implying the selected patients do not represent a biased group.

**Table 5.3:- Comparison of nonlinear mixed effects modelling for the two datasets:- reduced dataset (only patients where individual fits were feasible) & inclusive dataset (reduced dataset plus the 35 patients with missing data or early peaks).**

Parameter	Nonlinear mixed effects modelling (n=152)		Nonlinear mixed effects modelling (n=117)	
	Estimate (SE)	90% PI	Estimate (SE)	90% PI
a	1.32 (0.06)	0.46,2.18	1.31 (0.06)	0.60,2.02
PMR	8.24 (0.39)	5.52,12.29	8.20 (0.37)	6.55,10.27
c	0.44 (0.03)	0.19,1.01	0.50 (0.04)	0.23,1.10
Period	2.35 (0.02)	-	2.36 (0.02)	-
k	0.37 (0.10)	-	0.38 (0.09)	-
$\omega_a$	0.520		0.432	
$\omega_{PMR}^*$	0.243		0.137	
$\omega_c^*$	0.503		0.484	
$\omega_{period}$	-		-	
$\omega_k$	-		-	
Residual SD - $\sigma_e$	0.503		0.485	

SD - standard deviation; SE – standard error; PI – prediction interval;

$\omega$  - inter-patient standard deviation for the parameters a, PMR, c, period and k;

\* - log-normal error model.

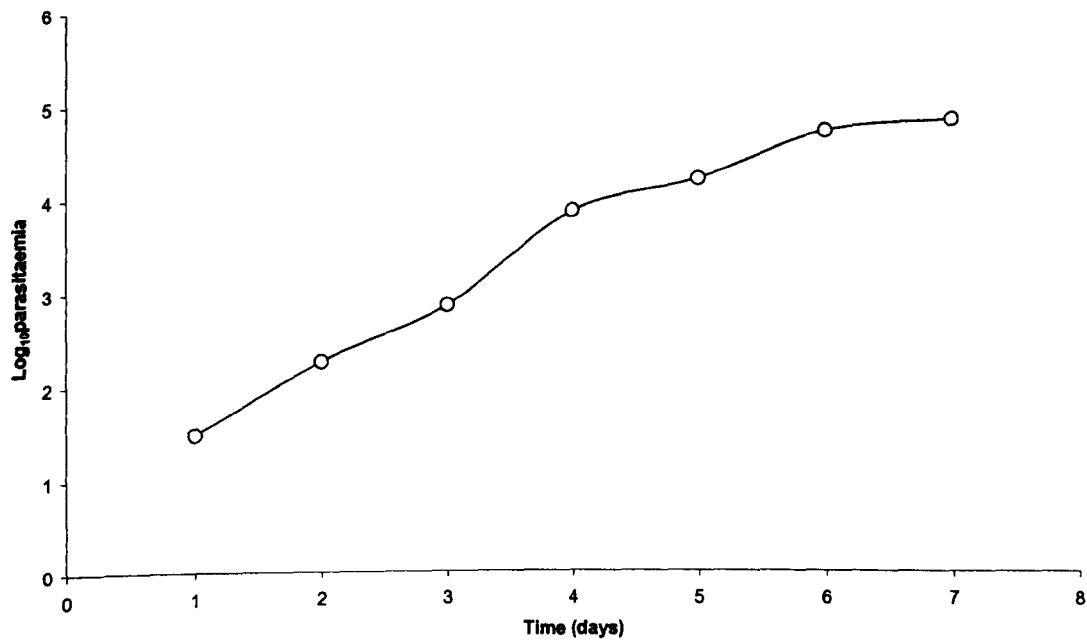
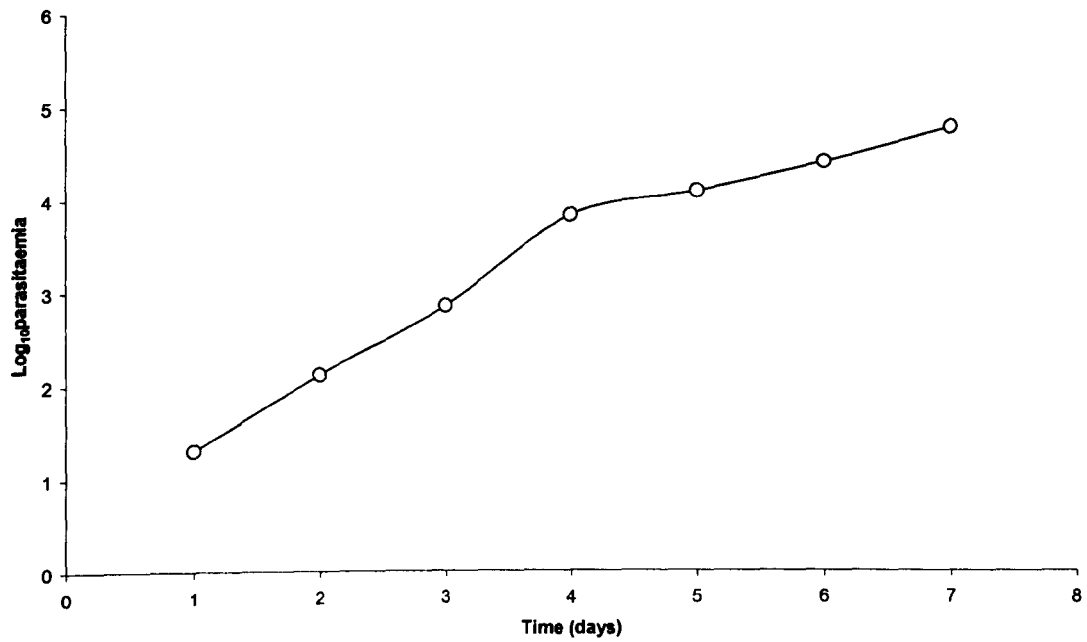
Mathematical equation fitted to data:-

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t) + c \times \sin(\frac{2\pi}{period} .t + k)$$

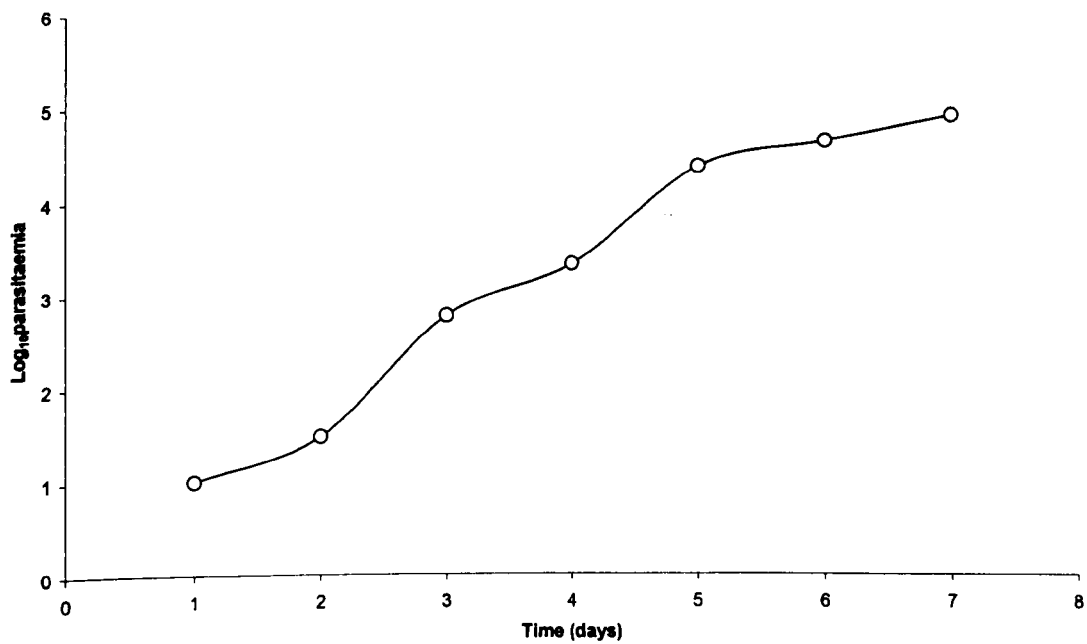
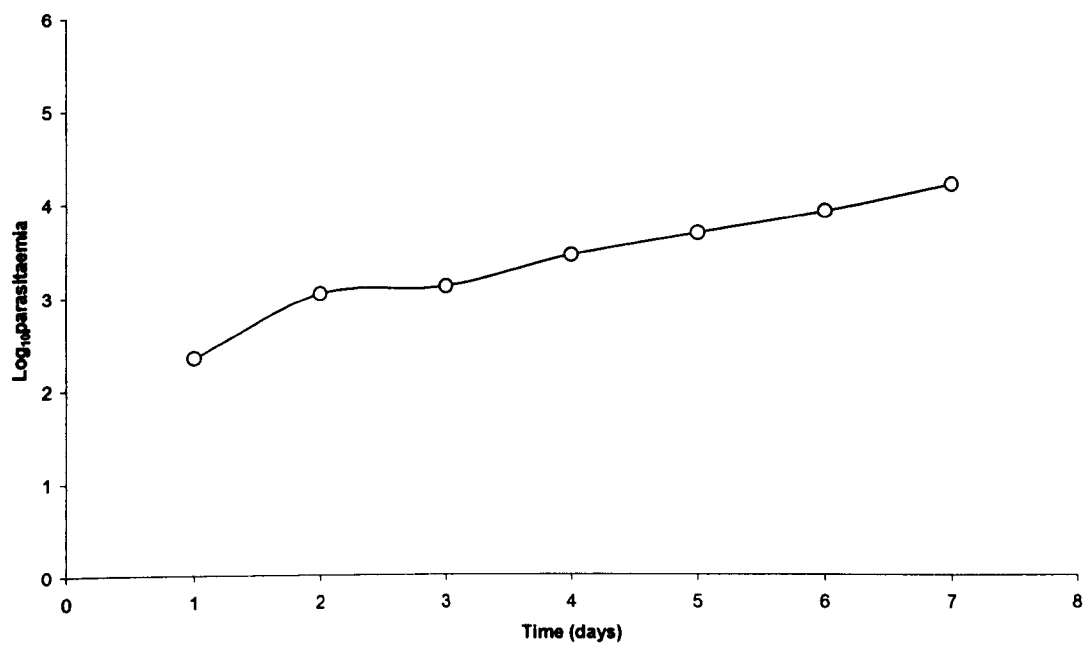
### 5.3.2 Non-oscillating data

Sixty of the patients were observed to have parasite versus time profiles that did not oscillate (see Figures 5.5a-5.5d for four examples). These patients probably had asynchronous infections, that is, the age distribution of all the parasitised cells (circulating and sequestering) is almost uniformly distributed (White et al. 1992a). Equation 5.1 was fitted to the data to obtain estimates of PMR using nonlinear mixed effects modelling.

Table 5.4 presents the results. The patients’ with non-oscillating parasitaemia versus time profiles were observed to have slightly higher PMRs compared to those patients’ with oscillating profiles (8.84 (90% PI: 5.59,14.00) versus 8.24 (5.52,12.29)).



**Figures 5.5a-b.** Parasitaemia versus time profiles for patients with an asynchronous parasite population.



**Figures 5.5c-d.** Parasitaemia versus time profiles for patients with an asynchronous parasite population.



**Table 5.4:- Nonlinear mixed effects modelling results for the 60 patients with non-oscillatory parasitaemia-time profiles.**

Nonlinear mixed effects modelling		
Parameter	Estimate (SE)	90% PI
a	1.38 (0.07)	0.72, 2.04
PMR	8.84 (0.58)	5.59, 14.00

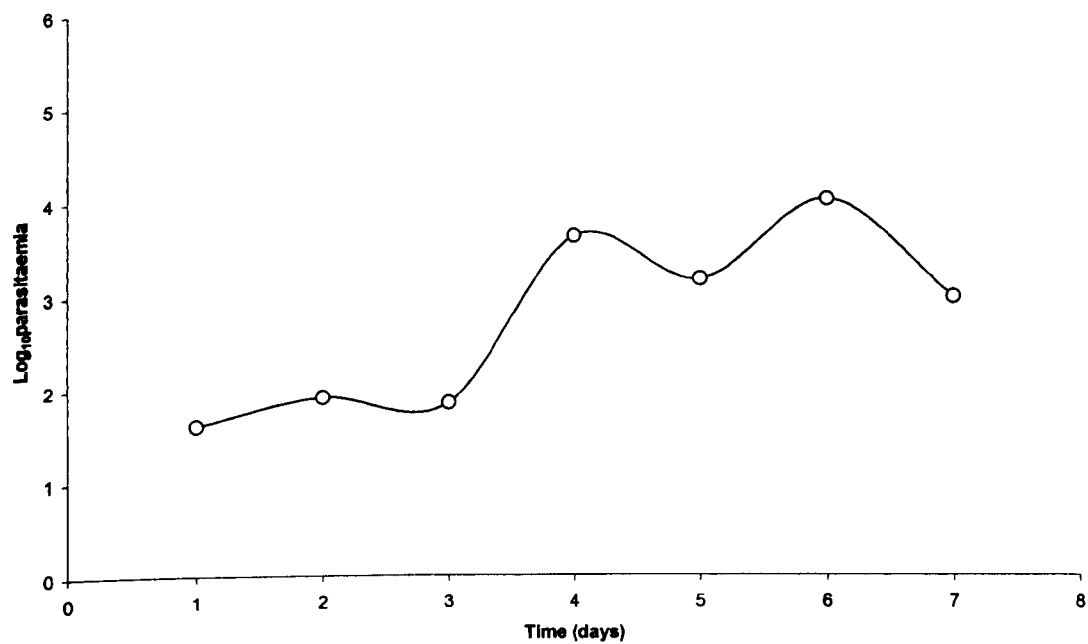
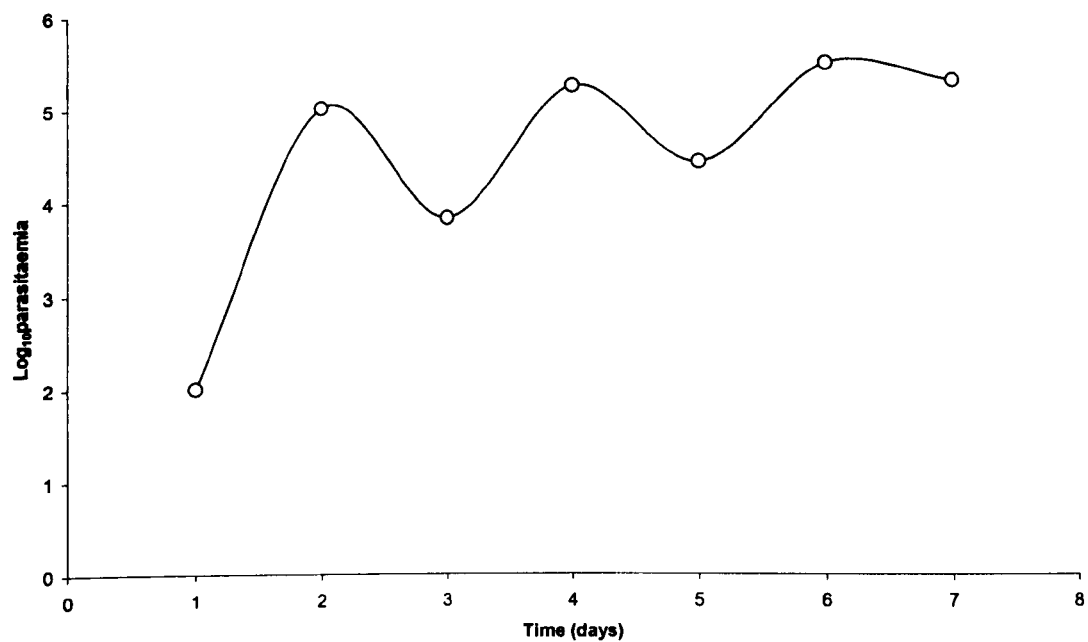
SE – standard error; PI – prediction interval.

Mathematical equation fitted to data:-

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t)$$

### 5.3.3 Amplitude of oscillation fluctuating

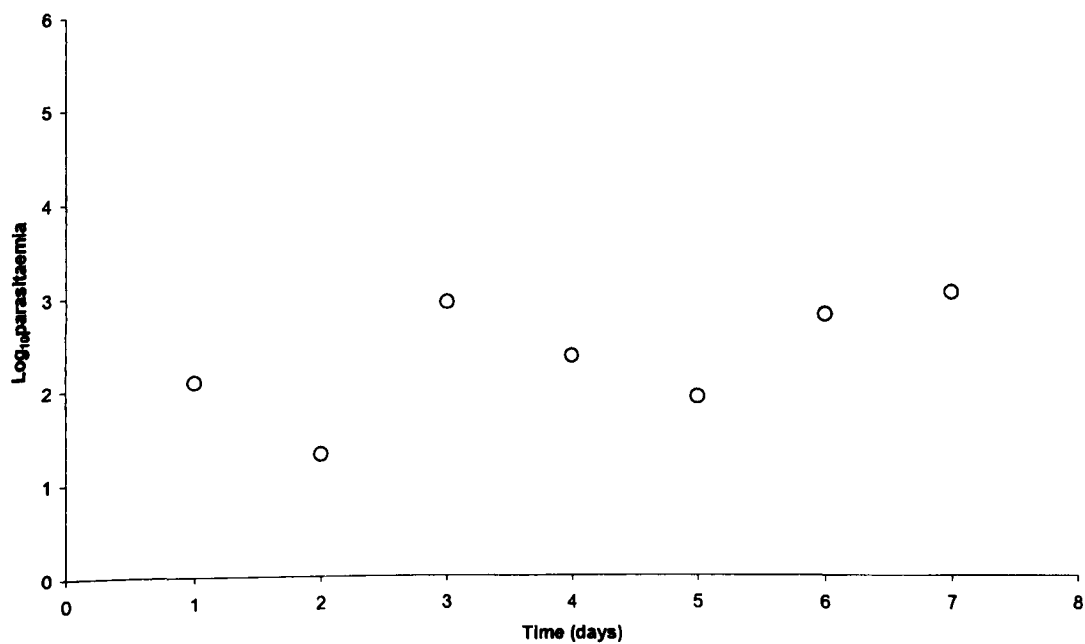
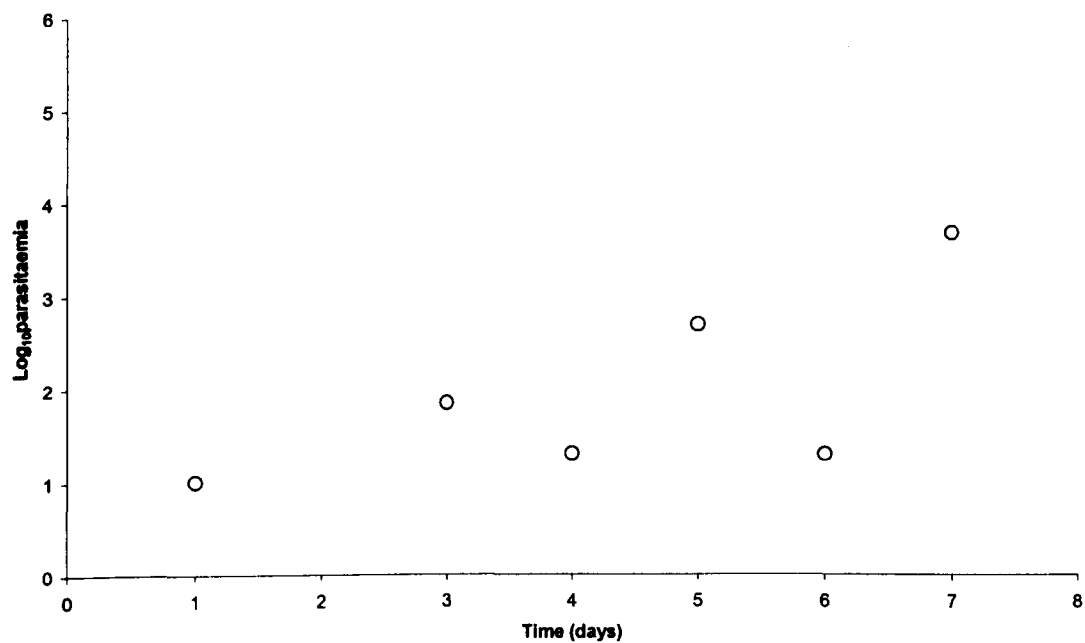
Figures 5.6a and 5.6b represent two parasitaemia-time profiles for patients’ with a fluctuating amplitude of the oscillation around the loglinear rise in parasitaemia. In Figure 5.6a the rise and fall of the parasitaemia declines with time (i.e. the amplitude of the sine wave decreases with time). A high parasitaemia is reached rapidly reflecting a high PMR and thereafter the parasitaemia stabilises. For this patient the distribution of the age of parasites in their blood could be skewed towards younger parasites. In Figure 5.6b, the initial rise and fall in parasitaemia becomes more abrupt with time, suggesting the age distribution of the parasites infecting this patient is skewed towards older parasites.



**Figures 5.6a-b.** Two examples of patients with parasitaemia versus time profiles that cannot be fitted with a rising sine wave.

#### 5.3.4 Shape very different from structural model

The seventeen parasitaemia-time profiles were a mixture of shapes. A few of them appeared to represent highly synchronous infections (i.e. the majority of parasites are the same age). The rise and fall in the parasitaemia was abrupt (see Figure 5.7 for two examples). The peaks of the oscillations below in Figures 5.7a and 5.7b may represent nearly all the parasites in the body at the corresponding time. Sequestration of the circulating parasitised red cells leads to a dramatic downward change in the parasitaemia.



**Figures 5.7a-b.** Parasitaemia versus time profiles for two patients with a highly synchronous parasite population.

### 5.3.5 Covariates

Formal hypothesis testing of the effect of each of the covariates on the parameters of interest (PMR and period) was performed using nonlinear mixed effects modelling of data from the 152 patients described previously (see Table 5.3). As the parameter ‘period’ had little inter-patient variability, only relationships between the covariates and the parameter ‘PMR’ were investigated. Table 5.5 summarises the univariate effects of each covariate on the parameter ‘PMR’. Those infections acquired via sporozoites had PMR values that were not significantly different to those following inoculation of parasitised blood. The El Limon and Santee Cooper strains both had higher PMR values compared with the McLendon strain (absolute mean increase in PMR related to El Limon: 2.06 (95% CI: 0.63,3.49) and to Santee Cooper: 1.98 (95% CI: 0.53,3.43) when compared to McLendon strain (mean PMR=7.13)). The final population parameter estimates, the inter-patient variability, and the residual variability including the effect of parasite strain are given in Table 5.6. Including the covariate, ‘strain’, in the model reduced the inter-patient variability of the parameter ‘PMR’ from 0.24 to 0.20.

**Table 5.5:-Summary of results with incorporation of covariates into the base model (n=152 patients).**

	Parameter estimate	95% CI	Change in objective function	p-value
Effect of sporozoite induced infection on PMR	-0.30	-1.53,0.93	0.22	0.65
Effect of strain on PMR (n=146*)			10.89	<0.01
Mc Lendon (n=65)	Reference	-		
El Limon (n=41)	2.06	0.63,3.49		
Santee Cooper on PMR (n=40)	1.98	0.53,3.43		

CI – confidence interval

\* - Six of the patients were infected with the following strains: Colombia (n=2), Costa (n=1), So.Rhode (n=1), Strain “7” (n=1) and Thailand (n=1).

**Table 5.6:- Final estimates of parameters from the optimal model.**

Parameter	Nonlinear mixed effects modelling (n=146)	
	Estimate (SE)	90% Prediction Interval
a	1.32 (0.06)	0.45,2.19
PMR (McLendon)	7.13 (0.44)	5.13,9.91
Factor for El Limon	2.06	
Factor for Santee Cooper	1.98	
c	0.44 (0.03)	0.19,1.00
Period (days)	2.36 (0.02)	-
k	0.37 (0.10)	-
$\omega_a$	0.53	
$\omega_{PMR}^*$	0.20	
$\omega_c^*$	0.50	
$\omega_{period}$	-	
$\omega_k$	-	
Residual SD - $\sigma_e$	0.503	

SE – standard error; SD – standard deviation;

$\omega$  - inter-patient standard deviation for the parameters a, PMR, c, period and k;

\* - log-normal error model.

Mathematical equation fitted to data:-

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t) + c \times \sin\left(\frac{2\pi}{period} \cdot t + k\right)$$

### 5.3.6 Association between PMR and both observed peak parasitaemia and amplitude of sine wave

There was a significant strong positive correlation between PMR and the observed maximum parasitaemia in the first 7 days (Spearman rank correlation coefficient = 0.532,  $p < 0.001$ ). A significant negative association was observed between PMR and amplitude of the sine wave (Spearman rank correlation coefficient = -0.184,  $p = 0.026$ ).

5.3.7 Association between PMR and the different patients groups omitted from the analyses

Of the 328 patients, 176 were not included in the above analyses. These patients comprise of four different groups: treated within the first 7 days (n=83), no oscillation in the peripheral parasite-time profile (n=60), variable amplitude in the peripheral parasite-time profile (n=16), and shape very different from the structural model (n=17). Each of these patient groups may influence the PMR and by excluding them may result in a biased estimate of the PMR. Therefore the simple loglinear equation (i.e. equation 5.1) was fitted to the parasite data of all 328 patients using nonlinear mixed effects modelling. For the patients treated within the first 7 days only the parasite counts recorded before treatment were included in the modelling. Table 5.7 presents the results. The population mean estimate for PMR is 7.7 slightly less than the estimate observed when fitting equation 5.2 to the subset of 152 patients. When investigating the effect of the different patient groups on the estimate of the PMR, those patients treated during the first 7 days and those with non-oscillating parasite-time profiles had significantly higher PMRs whereas the patients with a parasite-time profile that was very different from the structural model had significantly lower PMRs (Table 5.8).

**Table 5.7:- Nonlinear mixed effects modelling results for all 328 patients.**

Parameter	Estimate (SE)	90% Prediction Interval
a	1.46 (0.04)	0.69, 2.23
PMR	7.70 (0.29)	4.70, 12.61
$\omega_a$	0.47	
$\omega_{PMR}^*$	0.30	
Residual SD - $\sigma_e$	0.633	

SE – standard error; SD – standard deviation;

$\omega$  - inter-patient standard deviation for the parameters a and PMR;

\* - log-normal error model;

Mathematical equation fitted to data:-

$$\log_{10} Y = a + (0.5 \times \log_{10} PMR \times t)$$

**Table 5.8:- Summary of covariate analysis of all 328 patients.**

Group	PMR estimate	95% confidence interval
Inclusive (n=152)	Reference	-
Treat (n=83)	5.93	4.05, 7.81
No_osc (n=60)	1.60	0.50, 2.70
Var_amp (n=16)	0.52	-1.22, 2.26
Shape_diff (n=17)	-2.96	-3.96, -1.96

‘Inclusive’ – the patient data modelled successfully by equation 5.2 using nonlinear mixed effects modelling;

‘Treat’ – treated in the first seven days;

‘No\_osc’ - no oscillation in the parasitaemia-time profile;

‘Var\_amp’ - variable amplitude of the oscillation in the parasitaemia-time profile;

‘Shape\_diff’ - shape very different from the structural model (i.e. an oscillatory profile around an exponential rise).

#### 5.3.8 Assessment of information in the experimental design

To calculate the Fisher information matrix for the above model and experimental design, the final estimates of the population parameters, inter-patient variability and intra-patient variability from mixed effects modelling plus the sampling times (day 1, 2, 3, 4, 5, 6, and 7) and number of samples for each patient (approximately 7) were investigated using a programme written for the software application MATLAB (Retout et al. 2001). The coefficient of variation for the population parameter estimates and estimates of inter-patient variability were reasonable (ranges of 0.6 % - 12.7% for population parameter estimates and 16 - 44% for inter-patient variability estimates) except for the inter-patient variability of ‘k’ (CV=301%). The parameter ‘k’ is included in the model to account for patients having their first detectable count parasite at different stages of the parasite cycle. It is not a parameter of primary importance. The results indicate that the other four parameters and their respective inter-patient variability are estimated reasonably from the sampling times (parasite counts recorded daily) of these data.



## 5.4 Discussion

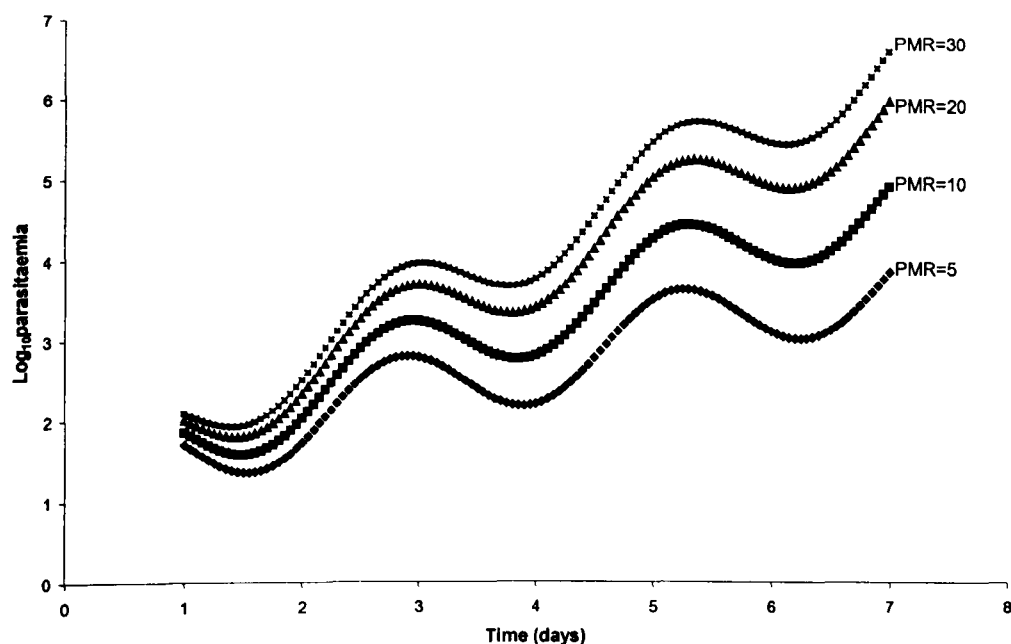
The majority of intra-host malaria models have no strong similarity to the natural infection observed in an individual (Molineaux and Dietz, 1999). Most of the intra-host malaria models (Gravenor et al. 1995; Hetzel and Anderson, 1996; Austin et al. 1998) are based on the model by Anderson et al. (1989) which is a set of four coupled differential equations representing the rate of change in uninfected erythrocytes, parasitised erythrocytes, merozoites and immune effectors over time. The data that were simulated from the above models were never formally compared to observed data. Furthermore the Anderson model cannot yield simultaneously a realistic value for the number of parasitised erythrocytes produced per schizont and the number of merozoites produced per schizont. Consequently an empirical model was chosen to fit the data from non-immune or partially immune adults receiving *P. falciparum* for the treatment of neurosyphilis. The advantage of the model chosen was that two of the parameters represented important biological measures, parasite multiplication rate every 48 hours (PMR) and length of parasite life cycle (period).

### 5.4.1 Summary of findings

The patient parasitaemia versus time profiles demonstrated huge variability across patients. Fitting the data separately for each individual or by using nonlinear mixed effects modelling gave similar mean population parameter estimates but varying estimates for inter-patient variability of some of the parameters. As expected the inter-patient variability for the parameters 'a' and 'PMR' were over-estimated by the STS method compared to GTS and nonlinear mixed effects modelling. Analysis of pharmacokinetic experimental data has also shown the STS approach to overestimate systematically the inter-individual variability of the parameters (Sheiner and Beal, 1981). Nonlinear mixed effects modelling estimates are not contaminated with the error involved in the estimation of individual parameters.

The mean population estimate of 'PMR' was approximately 8 and varied across patients. This differs from the Figure of 12.3, reported by Gravenor et al. (1995) which was obtained from simple loglinear regression of the initial growth phase (defined as that period until the maximum parasitaemia during the first week of detection in the blood). In this chapter eighty-three of the patients were excluded from the analysis because they received antimalarial treatment during the first seven days of the infection whereas Gravenor et al. (1995) analysed all patient data and only excluded the parasite counts recorded after the administration of quinine. This implies that the estimate of the PMR could be biased downwards. However when fitting equation 5.1 to the data of all 328 patients (i.e. including the pre-treatment parasite count data from the 83 patients treated within the first 7 days and the parasite count data from the 93 patients where equation 5.2 could not fit the data) the population mean estimate of PMR remained approximately 8.

PMR was observed to be highly dependent on the *P. falciparum* strain. The strains given to the patient are nested under institution (El Limon and Santee Cooper strains in Georgia, McLendon strain in South Carolina). Consequently the differences in PMR could also be confounded by institutional differences in treatment practices. For example, at the South Carolina institution, there might have been a more conservative criterion for determining the patients requiring treatment to control the infection which would explain the lower PMRs. However at the Georgia location there was a significantly higher percentage of patients treated compared to South Carolina (31% versus 20% respectively,  $p$ -value=0.042). This finding favours the explanation that the observed differences in PMR result from intrinsic multiplication differences between the different *P. falciparum* strains as opposed to the different institutions. Figure 5.8 illustrates the change in predicted parasitaemia for different PMR values.



**Figure 5.8.** Predicted parasitaemia for varying PMRs

The mean population estimate of the length of the parasite life cycle was approximately 55 hours (2.3 days) which is greater than the generally quoted 48 hours. The periodicity of the sine wave is highly dependent on the exact timings of the recording of parasite counts. Parasite counts were determined daily and for this analysis they were assumed to be exactly 24 hours apart. However blood films were made each morning usually between 8 and 10 am. On any given day, the timing of the blood film could vary by several hours from the day before to the day after.

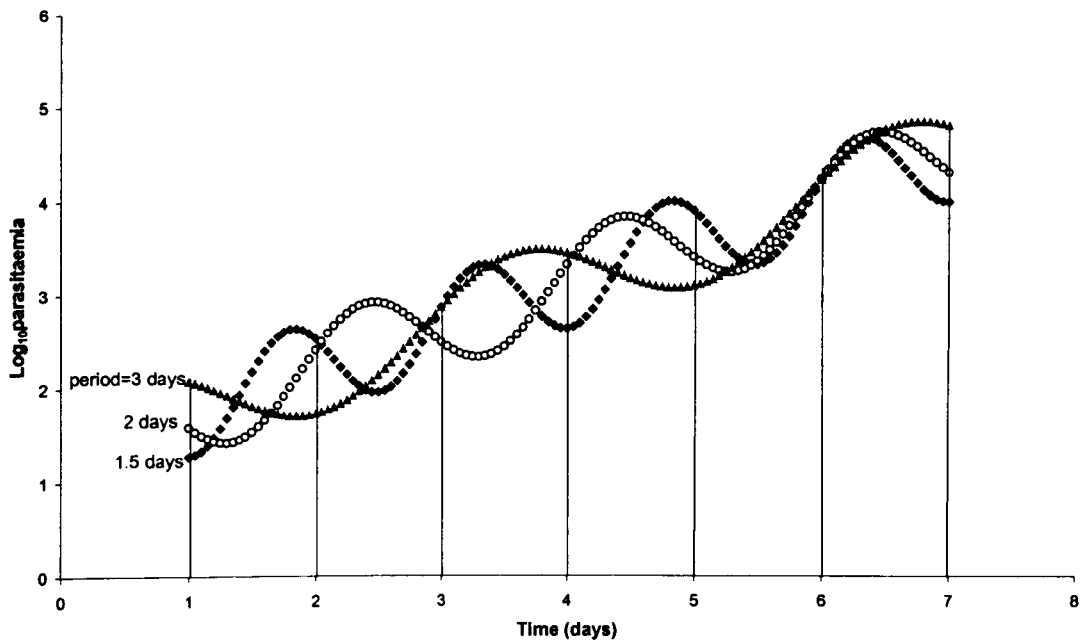
Given that considerable variation was observed across patients and parasites for PMR, future intra-host models and pharmacokinetic-pharmacodynamic models that include parasite multiplication must incorporate both variation among parasites and individual hosts. In these induced *P. falciparum* infection studies the population estimate for PMR was approximately eightfold per two day life cycle with a 90% prediction interval from 5.5 to 12.3. These values therefore represent the maximum values for zero drug effect in any study of antimalarial pharmacodynamics in non-immune patients.

#### 5.4.2 Limitations of the model

The parasitaemia versus time profile does not continue to rise indefinitely. It is usually contained by the host. The control mechanism intervenes abruptly reducing the PMR from approximately 8 fold per cycle to a value of one or less. The model used for this paper will predict the parasitaemia continues to rise after seven days not reflecting the true pattern of the infection. Thus the predictive values of this model are confined to the unrestrained expansion phase of the infection and cannot be extrapolated past seven days.

#### 5.4.3 Limitations of the data

The sampling interval for the parasite count data was one day. For a time series with a one-day sampling interval the highest frequency, termed 'Nyquist frequency', that can be restored is 0.5. In other words the minimum period (i.e. parasite life cycle) that can be determined from the data is two days. With the current data, nonlinear mixed effects modelling did not produce a parameter to represent the inter-individual variability in the parameter 'period of parasite life cycle'. Therefore it is not possible to determine the probability of the period being less than 48 hours. Figure 5.9 presents predicted parasitaemia versus time profiles for varying periods of the sine wave. Vertical lines are drawn at daily intervals to highlight the limited data available for predicting the periodicity of the sine wave.



**Figure 5.9.** Predicted parasitaemia for varying periods of the sine wave.

Fluctuations in parasitaemia are caused by sequestration and ring form clearance (fall in parasite count), and later schizont rupture and meront reinvasion of erythrocytes (rise in parasite count). The inability of the model to fit adequately 93 of the individuals is not necessarily a result of model misspecification. It may be explained by discrepancies between the peripheral blood parasite count (detected microscopically) and the total parasite burden in *falciparum* malaria. The ratio of circulating to sequestering cells at any time is largely dependent on the synchronicity of the infection (White et al. 1992a). If the parasite age distribution is synchronous and from a single or dominant “brood”, then the observed parasitaemia increases with a rising sine wave pattern (Figures 5.3a & 5.3b). In highly synchronous infections the peripheral parasitaemia rises and falls dramatically (Figures 5.7a & 5.7b) whereas a completely asynchronous infection gives a rising straight line on a semi-log plot (Figures 5.5a-d). Parasites with an age distribution that is not normally distributed will have very different parasitaemia versus time profiles. Some infections consist of two or more discrete “broods” with different characteristics, giving

rise to an infinite range of possible profiles. White et al. (1992a) presented a model that contained just two parameters; the standard deviation of parasite age since merogony (schizogony) as a measure of synchronicity, and a multiplication factor for each 48 hour asexual life cycle. Svensson (2000) adjusted for synchronicity by including a parameter that represented the time for one life cycle in the average parasite, that is parasite mean residence time. Although parasite mean residence time does not measure synchronicity of an infection it does present a way of taking account of the variation across patients resulting from synchronicity. In this chapter the amplitude of the sine wave of equation 5.2 represents empirically the synchronicity of the infection. Patients with very large amplitudes have highly synchronous infections whereas moderately synchronous infections have amplitudes close to zero. Aysnchronous infections could not be fitted by equation 5.2 because there was no oscillation in the profiles. The amplitude did not differ between patients infected with different *P. falciparum* strains. *In vivo* it is not possible to measure the synchronicity of an infection as not all parasite stages are present in the peripheral blood. Therefore to calculate the total parasite burden and the number of parasites within each stage one needs to extrapolate from data collected describing the distribution of parasite stages in the peripheral blood. Even with these proxy distributions of parasite age it is still difficult to mechanistically quantify synchronicity.

The mathematical model presented in this chapter can only be validated in the relatively narrow range of detectable parasitaemias (i.e. parasitaemias above 10 / $\mu$ l or a total parasite burden of  $> 5 \times 10^7$  parasites). Additionally the parasitaemias recorded may be inaccurate due to small counting errors; this error increases with decreasing numbers of parasites per slide.

## **6. SEQUENTIAL PHARMACOKINETIC/PHARMACODYNAMIC MODELLING FOR MEFLOQUINE**

### **6.1. Introduction**

Information regarding the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug helps provide the clinician with optimal dosing strategies. Recent statistical methodology enables concurrent mathematical modelling of the kinetics and dynamics. There are two approaches to this type of modelling, sequential PK-PD modelling (see section 1.2.3.1) or simultaneous PK-PD modelling (see section 1.2.3.2). In brief, for the sequential approach population PK modelling of the concentration-time data is performed initially and from this modelling concentrations are predicted which correspond to the times at which the PD measurements were taken. Population PD modelling is then performed using the PD-time data and the predicted concentrations. Whereas for simultaneous modelling both the PK and PD models are fitted together to all observed PK and PD measurements.

Population PK-PD modelling has already been applied successfully to a number of different medical conditions. Four examples, from Phase III trials, are given by Machado et al. (1999) where the results of the population PK-PD modelling assisted regulatory decision-making. Population modelling has also been applied to data from preclinical PK and toxicokinetic studies, dose titration studies, and the integration of PK-PD into disease progression models.

For the antimalarial drug, mefloquine, little work has been done using the population approach to investigate how the drug concentrations relate to the therapeutic effect.

Svensson (2000) applied population PK-PD modelling to patients with falciparum malaria receiving one of three different treatments: oral artemisinin for 3 days followed by mefloquine on day 4; oral artemisinin for 3 days plus mefloquine on day 1; or a single dose of mefloquine. The mechanism-based PD model successfully described the antimalarial efficacy of artemisinin, mefloquine and a combination of the two drugs.

In this chapter sequential PK-PD modelling was performed on PK and PD data collected from patients receiving mefloquine monotherapy for the treatment of uncomplicated *P. falciparum* malaria during 1990-1992. Individual specific PK parameter estimates obtained from the population PK modelling performed in Chapter 3 were used to predict concentrations corresponding to the times at which the PD measurements, that is parasite counts, were taken. The mechanistic PD model derived in Chapter 4 was the mathematical model chosen to represent the change in parasite count over time for the sequential PK-PD modelling.

## **6.2 Methods**

### **6.2.1 Study population**

PK and PD data were collected prospectively from patients receiving mefloquine monotherapy as part of trials conducted between 1990 and 1992. The patients were from the Karen ethnic minority and lived in camps for displaced persons situated along the western border of Thailand in an area of low and seasonal malaria transmission (Luxemburger et al. 1996). Health care in these camps is provided by Médecins Sans Frontières (MSF).

Patients who came to the clinics were enrolled into prospective studies of antimalarial treatment if they had symptoms and a microscopically confirmed infection with asexual



stages of *P. falciparum* in the peripheral blood. The exclusion criteria for these studies were the following; weight less than 8 kg, quinine administration within the previous 7 days, or mefloquine or sulfadoxine-pyrimethamine within the previous 14 days, pregnancy, signs of severe or concomitant disease requiring hospital administration (World Health Organisation, 1990).

All patients received one of the two following mefloquine (Lariam, 250 mg base tablets, Roche Pharmaceuticals) dosing regimens: 25 mg/kg (single dose) or 25 mg/kg (split dose: 15 mg/kg on day 0 followed by 10 mg/kg 24 hours later).

Informed consent was obtained from the patients or their parents or guardians. All studies were approved by the Ethical Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, and by the Karen Refugee Committee, Mae Sod, Thailand.

#### 6.2.2 Pharmacodynamic model

The variable chosen to represent therapeutic response was the number of parasites recorded in a malaria smear. Malaria parasite counts were determined on Giemsa-stained thick films as the number of parasites per 500 white blood cells, and converted to parasitaemia by assuming a white blood cell count of 8000 / $\mu$ l. Patients were examined daily thereafter until they became aparasitaemic, and they were then seen once a week for at least 4 weeks. If the blood smear was positive on day 7, and the patient was asymptomatic, smears were taken again on days 8 and 9. If still positive, the case was considered a failure and retreated. Symptomatic patients with a positive blood smear on day 7 were considered failures and retreated immediately. Patients whose blood smears became positive between days 9 and 28 were also considered failures and retreated irrespective of symptoms.

Parasitaemia was converted to 'Total parasite burden' by multiplying the parasitaemia (/μl) by  $(5 \times 10^6)/70$  (blood volume μl/kg) and the body weight (kg) of the patient.

The mathematical model chosen to represent 'Total parasite burden' over time was the following mechanism-based differential equation (see section 4.2.1 for more detail):-

$$\frac{dP_{ij}}{dt} = a.P_{ij} - [k_1 \left( \frac{C_{ij}^\gamma}{C_{ij}^\gamma + C_{50}^\gamma} \right)].P_{ij} \quad \text{-----} \quad (6.1)$$

where

$P_{ij}$  – total parasite burden for  $i^{\text{th}}$  subject at  $j^{\text{th}}$  time,

$C_{ij}$  – mefloquine level for  $i^{\text{th}}$  subject at  $j^{\text{th}}$  time,

$t$  – time (days),

$P_0$  – total parasite burden at admission,

$k_1$  – rate constant of parasite killing,

$C_{50}$  – drug concentration that kills 50% of the parasite,

$a$  - growth rate constant of the parasite,

$\gamma$  - slope of concentration effect curve.

Following treatment with mefloquine, the average parasitaemia-time profile is that of a lag phase, during which the population mean parasitaemia remains unchanged (White, 1997). The explanation for this is the stage-specific killing action of mefloquine. Mefloquine mainly kills trophozoites, that is, the older parasites that are not visible to the microscopist (Geary et al. 1989). Thus, a time lag of one day was introduced into the model.

Another alteration to the model was introduced. When the predicted total parasite burden dropped below 10 parasites the programme was set so that the predicted parasite burden

remained <10 parasites and the patient was predicted as cured. This was implemented to avoid equation 6.1 predicting that a patient had recrudesced even though their minimum predicted total parasite burden was less than 10 parasites (i.e. radical cure).

The mefloquine levels for each subject at each time point where parasitaemia was recorded were simulated using the individual specific PK parameter estimates obtained from the population PK modelling executed in Chapter 3. The use of the predicted, rather than observed, concentrations may reduce attenuation bias in estimating the population PD parameters, as observed concentrations are measured with error.

The equations used for the simulations are given below:-

The subject's given a split dose – 15 mg/kg on day 0, 10 mg/kg on day 1.

$$C_i = \left[ \frac{7 \times 15000}{(7 \times V_i) - CL_i} \times (e^{-\frac{CL_i}{V_i} \cdot t} - e^{-\gamma \cdot t}) \right] + \left[ \frac{7 \times 10000}{(7 \times V_i) - CL_i} \times (e^{-\frac{CL_i}{V_i} \cdot (t-1)} - e^{-\gamma \cdot (t-1)}) \right] \quad \text{----- (6.2)}$$

The subject's given a single dose – 25 mg/kg on day 0

$$C_i = \left[ \frac{7 \times 25000}{(7 \times V_i) - CL_i} \times (e^{-\frac{CL_i}{V_i} \cdot t} - e^{-\gamma \cdot t}) \right] \quad \text{----- (6.3)}$$

The mechanism-based differential equation (equation 6.1) cannot be integrated analytically and instead was integrated numerically using a FORTRAN NAG (Numerical Algorithms

Group, Oxford, UK) routine, 'DO2EAF', that employs a Runge Kutta method (Farlow, 1994) to numerically integrate the differential equation.

### 6.2.3 Statistical model

A lognormal error model was used to characterise the residual intra-individual error:

$$\log_e \left( \int \frac{dP_{ij}}{dt} \right) = \log_e \left( \int \frac{dP_{pij}}{dt} \right) + \varepsilon_{ij}$$

In which  $P_{ij}$  is the  $j^{\text{th}}$  observed parasite count for the  $i^{\text{th}}$  individual and  $P_{pij}$  is the predicted parasite count for the  $i^{\text{th}}$  individual.  $\varepsilon_{ij}$  is the residual intra-individual error term and is assumed to be randomly Normally distributed with zero mean and variance  $\sigma^2$ .

The inter-individual variability in the PD parameters was modelled according to an exponential variance model; that is, it was assumed that the PD parameters are log-normally distributed:

$$\text{e.g.} \quad k_{1i} = k_1 \times \exp(\eta_i^{k1})$$

where  $k_{1i}$  is the  $k_1$  parameter of the  $i^{\text{th}}$  individual,  $k_1$  is the population mean parameter, and  $\eta_i^{k1}$  expresses the random difference between  $k_{1i}$  and  $k_1$ . The values for  $\eta_i^{k1}$  are normally distributed with zero mean and variance  $\sigma_{k1}^2$ .

### 6.2.4 Data analysis

The PD model was fitted to the data with the nonlinear mixed effects modelling programme NONMEM (version IV) using a population approach (Beal and Sheiner, 1979). The programme applies the first-order conditional estimation method (Beal and

Sheiner, 1992) to obtain the population PD parameters, the inter-individual variability in these parameters and the residual intra-individual variability. The best PD model was assessed using the NONMEM objective function and inspection of scatterplots of the residuals versus the predicted values. A difference in the objective function of greater than 3.84 ( $\chi^2$  value for 1 degree of freedom) for an additional parameter in the model was interpreted as a significant change in the fit (p-value<0.05).

### **6.3 Results**

One hundred and fifty-two patients with acute falciparum malaria between 1990 and 1992 were available for analysis. Of these 104 were cured (patient did not recrudescence during 28 days of follow-up) and 33 failed. The remaining 15 patients were last seen with a negative malaria smear on the following days: day 7 (1 patient), day 8 (1), day 14 (6) and day 21 (7). The demographic characteristics of the patients are given in Table 6.1.

The limit of detection of parasites microscopically is 50 parasites per  $\mu\text{l}$  or a 'Total parasite burden' of  $2.5 \times 10^8$ . Parasite counts that were recorded as zero were therefore excluded since the parasitaemia could be anywhere between 0 and 50 parasites per  $\mu\text{l}$  (i.e. a parasite burden between 0 and  $2.5 \times 10^8$  parasites).

**Table 6.1:- Demographics and admission characteristics of patients receiving mefloquine monotherapy (n=152).**

	Cured (n=104)	Failed (n=33)	Unknown (n=15)
Year of Study	1990 – N=44 1991 – N=60	1990 – N=9 1991 – N=24	1990 – N=10 1991 – N=5
Males N (%)	56 (54)	25 (76)	8 (53)
Age (years) <sup>a</sup>	16 (13.6-18.3) [1.5-54]	13 (9.3-16.7) [0.8-51]	15.6 (8.2-23.0) [2.7-54]
< 5 N (%)	10 (10)	6 (18)	2 (13)
5-14 N (%)	52 (50)	15 (46)	7 (47)
>14 N (%)	42 (40)	12 (36)	6 (40)
Weight (kg) <sup>a</sup>	32.6 (29.7-35.6) [8-61]	29.2(23.3-35.1) [8-59]	31.5(22.2-40.9) [10-55]
Primary Infection N (%)	85 (82)	19 (58)	9 (60)
Fever on admission N(%)	75 (72)	22 (67)	10 (67)
Prolonged history of fever N (%)*	65 (73)	21 (70)	12 (92)
Pure <i>P. falciparum</i> infection N (%)*	85 (86)	32 (100)	13 (93)
Haematocrit (%) <sup>a,*</sup>	35.6(34.2-36.9) [20-55]	33.3(30.2-36.4) [21-44]	30.6(26.2-35.0) [15-35]
< 30% N(%)	8 (11)	4 (25)	3 (30)
Parasite count per µl blood <sup>b</sup>	3014 (2096-4334) [50-101274]	5119 (2799,9360) [249-114660]	3968 (1326,11877) [243-146080]
>10,000 /µl N (%)	28 (27)	15 (46)	4 (27)

a – mean (95% CI) [range]

b – geometric mean (95% CI) [range]

\* - missing data for some patients (for haematocrit: cured n=72, failed n=16, unknown n=10).

**6.3.1 Patients defined as ‘cured’**

For the 119 patients that were cured (including the 15 patients who did not return for a blood smear on day 28) only 246 detectable parasite counts were recorded (median number per patient = 2, range = 1 - 4, see Table 6.2). These patients add little information for the modelling especially since a time lag of one day for the PD response to the drug was included in the model.

**Table 6.2 Number of detectable parasite counts recorded for patients defined as ‘cured’.**

Day	Frequency
0	119
1	73
2	42
3	12
Total	246

**6.3.2 Patients defined as ‘failure’**

The 33 patients who failed within 28 days had a total of 136 parasite counts (detected microscopically) recorded (median number per patient = 4, range = 2-7, see Table 6.3). Twenty of the patients received mefloquine as a split dose and 13 received mefloquine as a single dose.

**Table 6.3 Number of detectable parasite counts recorded for patients defined as ‘failure’.**

Day	Frequency
0	33
1	25
2	22
3	15
4	4
5	2
6	3
7	2
9	1
14	2
19	2
21	10
22	1
23	2
28	12
Total	136

#### 6.3.2.1 Population pharmacokinetic-pharmacodynamic modelling

Two of the parameters were entered into the model as fixed values:-

$a = 1.04$  /day, growth rate constant of the parasite.

This corresponds to the parasite multiplying and successfully re-invading 8 red cells every 2 days (48 hours). The value of 8 is the population mean parameter estimate of the parasite multiplication rate ( $PMR_{48}$ ) derived in Chapter 5.

$\gamma = 2.5$ , slope of concentration effect curve.

The value of 2.5 corresponds to the population mean parameter estimate derived from population modelling of the *in vitro* data in Chapter 4. It has been assumed that the slope of the concentration-effect curve *in vitro* is the same as *in vivo*.



The above two parameters were fixed because there is insufficient data to estimate all five parameters.

The parameters estimated from the data were the following:-

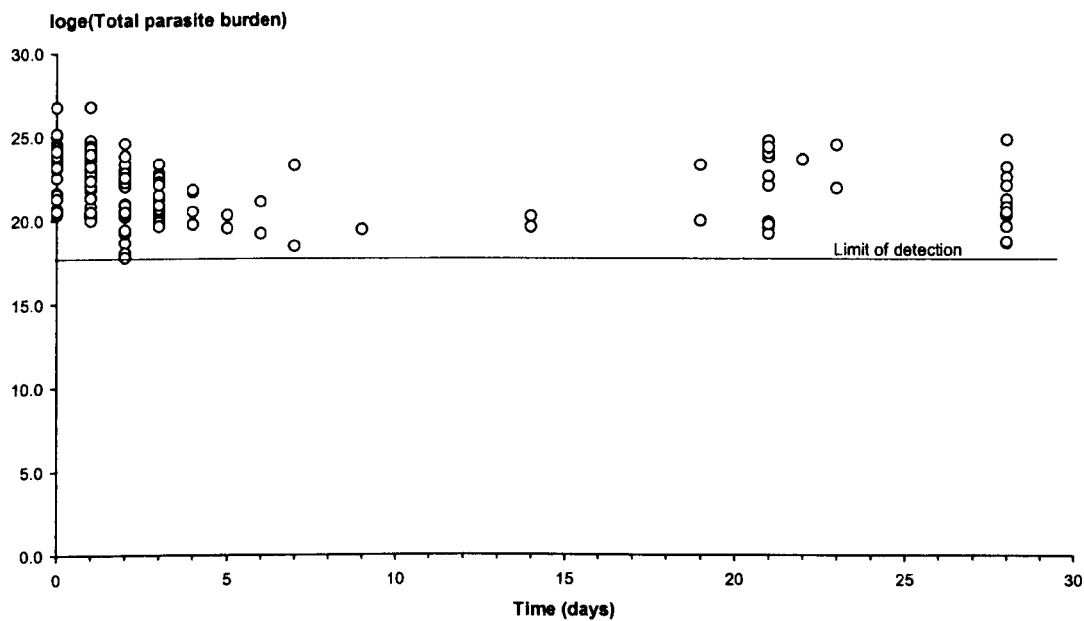
$P_0$  – total parasite burden at admission

$k_1$  – rate constant of parasite killing

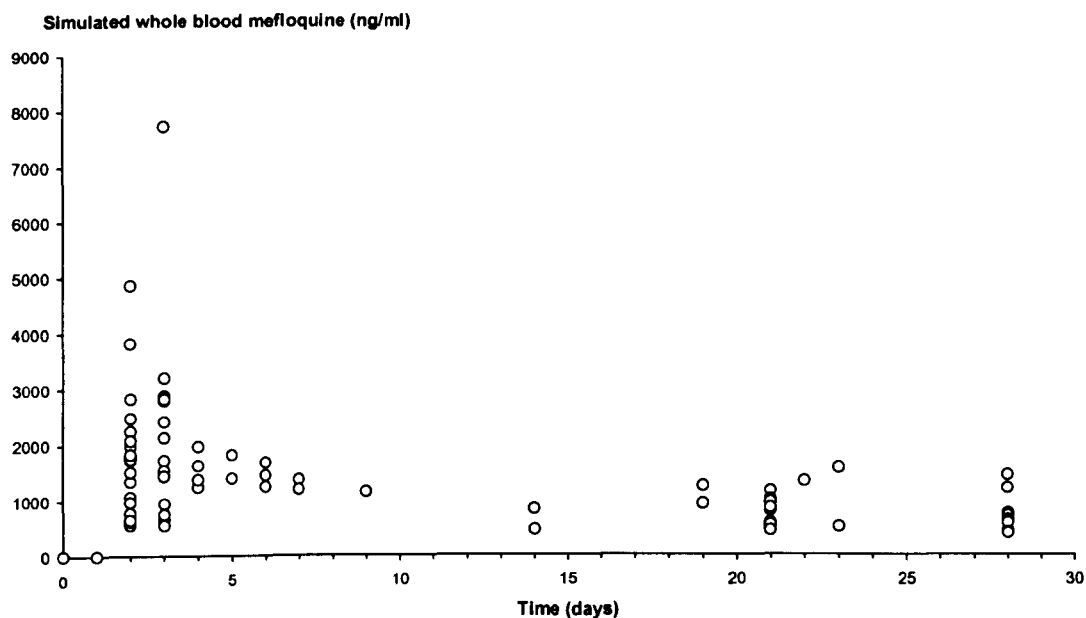
$C_{50}$  - drug concentration that kills 50% of the parasites

Using the posterior individual specific PK parameter estimates obtained from the population PK modelling performed in Chapter 3, whole blood mefloquine concentrations were simulated for the same times at which the individual's parasite counts were recorded. The mefloquine concentrations were simulated using equations 6.2 or 6.3 depending on whether the patient received a split or single dose respectively of mefloquine monotherapy. This gave 136 paired observations of parasite count and mefloquine concentration data. Figure 6.1a is a scatterplot of the observed total parasite burden versus time for all 33 patients. Figure 6.1b is a plot of the predicted mefloquine concentrations versus time. At day 1 the simulated mefloquine concentrations are zero because it has been assumed that there is a time lag of one day before a reduction is observed in the peripheral parasite count. Estimates of the population parameters and their variability obtained from the sequential PK-PD modelling are given in Table 6.4. The data did not permit estimation of the inter-individual variability of all three PD parameters, especially for the parameter  $C_{50}$ . The model that best fitted the data included inter-individual variability for both  $k_1$  and  $P_0$ . The population mean parameter estimate for  $k_1$  was observed to be 3.25 which equates to mefloquine reducing the number of parasites approximately 80 fold every 48 hours. The posterior individual estimates of  $k_1$  varied from 1.12 up to 4.53 (i.e. reducing parasites from 1.17 up to 1,111 fold every 48 hours). The population mean parameter estimate for the total parasite burden on admission ( $P_0$ ) was  $5.9 \times 10^9$  and the posterior individual

estimates varied from  $1.5 \times 10^9$  up to  $2.1 \times 10^{10}$ . The drug concentration which kills 50% of the parasites,  $C_{50}$ , was estimated to be 760 ng/ml. Therefore the average MIC *in vivo* for mefloquine against *P. falciparum* prevalent at the time of the study which caused recrudescence infections was 562 ng/ml (since  $MIC = C_{50} * ((k_1 - a)/a)^{-1/\gamma}$ , see section 4.2.2 of Chapter 4).



**Figure 6.1a.** The observed parasite counts (log transformed) over time for the 33 patients' who failed treatment during 28 days of follow-up.



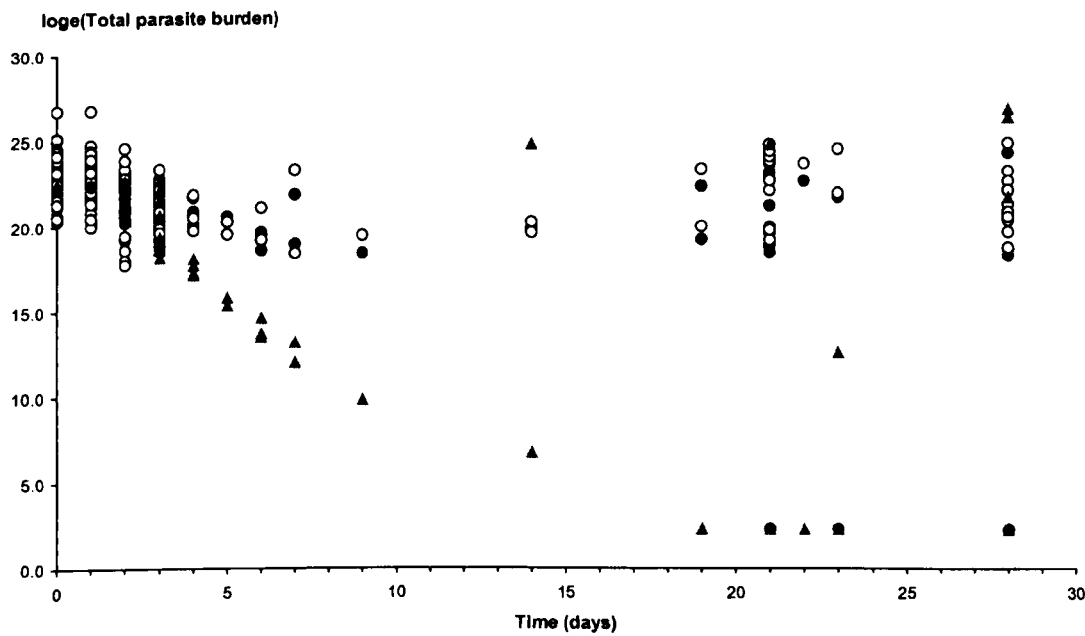
**Figure 6.1b.** The simulated whole blood mefloquine levels (ng/ml) at the time points where the parasite counts were recorded for the 33 patients' who failed treatment during 28 days of follow-up.

**Table 6.4:- Population pharmacodynamic parameters derived from sequential nonlinear mixed effects modelling of the parasite count and mefloquine concentration data.**

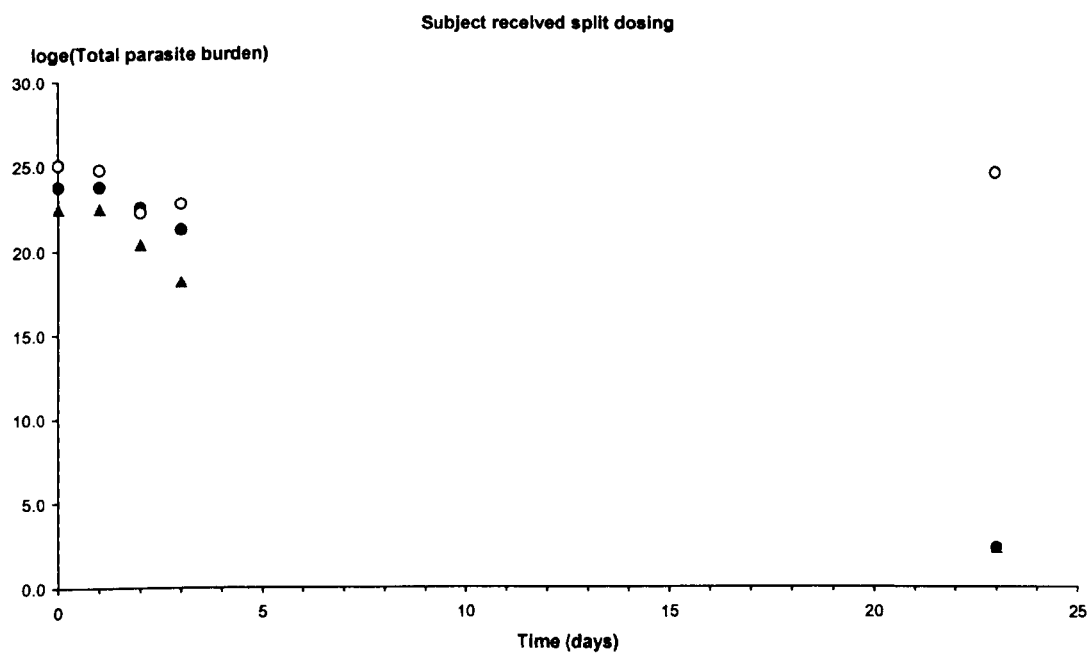
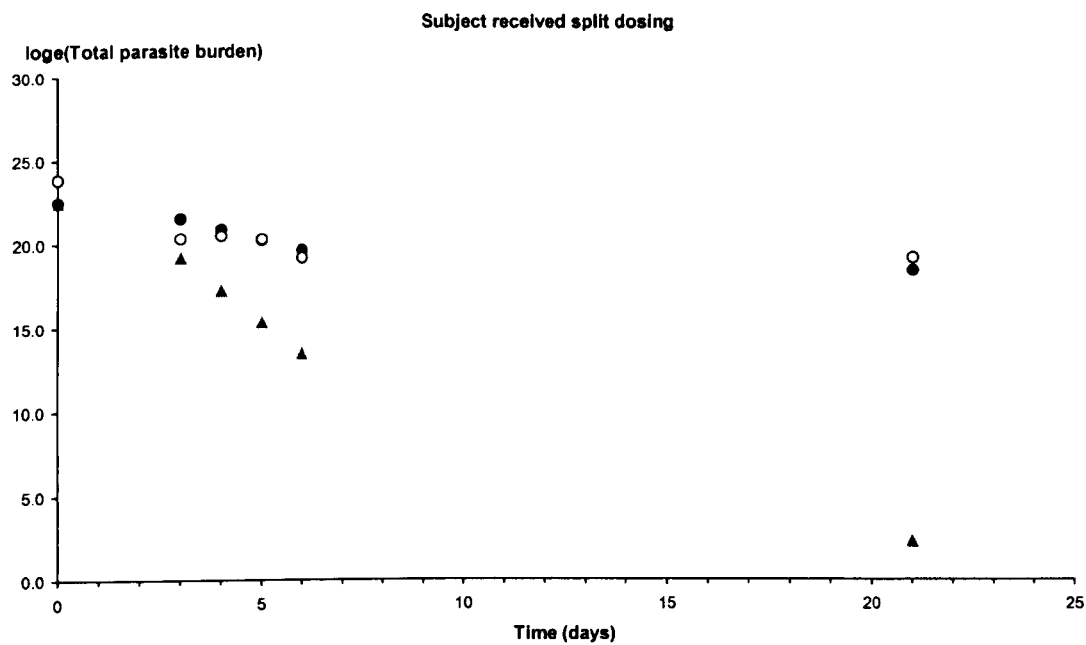
Parameter	Estimate (SE)	95% Prediction Interval
a (/day)	Fixed at the value of 1.04 (i.e. a PMR <sub>48</sub> of 8 fold)	
$\gamma$	Fixed at the value of 2.5	
Log <sub>e</sub> (P <sub>0</sub> )	22.5	
P <sub>0</sub>	5.9*10 <sup>9</sup>	1.07*10 <sup>9</sup> – 3.25*10 <sup>10</sup>
k <sub>1</sub> (/day)	3.25	1.67 – 6.33
C <sub>50</sub> (ng/ml)	760	
MIC <i>in vivo</i> (ng/ml)	562	
$\omega_{\log e P_0}$ *	0.87	
$\omega_{k_1}$ ^	0.34	
$\omega_{C_{50}}$	not estimated	
$\sigma_e$	1.46	
(residual error, units – log <sub>e</sub> P)		

\* - normal distribution; ^ - lognormal distribution

Figure 6.2 shows the observed parasite counts, population predicted values and individual predicted values versus time for the 33 patients who failed within 28 days. The population predicted values versus time for the 33 patients who failed within 28 days. The population predicted profile has at the same time point different predicted values of the total parasite burden. This is because the model (equation 6.1) does not just include parameters (e.g.  $k_1$ ) but also a variable that represents the mefloquine concentration ( $C_i$ ) for the  $i^{th}$  patient. For some patients ( $n=22$ ) the model predicts that the patient should have been cured but instead they actually failed. Figures 6.3a and 6.3b are examples of two patients. Figure 6.3a is of a patient that the population predicted values predicted the patient should have been cured while the individual predicted values predicted the patient failed. Figure 6.3b represents a patient that both the population and individual predicted values predict the patient is cured.



**Figure 6.2.** Observed parasite counts ( $\circ$ ), population predicted values ( $\blacktriangle$ ) and individual predicted values ( $\bullet$ ) versus time for the 33 patients' who failed treatment during 28 days of follow-up.



**Figures 6.3a & 6.3b.** Observed parasite counts (○), population predicted values (▲) and individual predicted values (●) versus time for two of the patients' who failed treatment during 28 days of follow-up.

#### 6.3.2.2 Relationship between pharmacodynamic parameters and covariates

The parameter estimates given in section 6.3.2.1 predict that 22 of the observed failures should have been cured. These 22 subjects include all 20 subjects that received a split dose and two subjects that received a single dose of mefloquine. These patients that the model incorrectly predicted were not different from the remaining 11 patients in age or admission parasite count but did have a significantly lower temperature on admission (see Table 6.5). The patients included in these studies had no evidence of receiving quinine within the previous 7 days, nor mefloquine or sulfadoxine-pyrimethamine within the previous 14 days. After 1994, on the basis of completed studies, these inclusion criteria were modified. Patients were only included in future studies and classified as a primary infection if no antimalarial medication had been given in the preceding 63 days. Historical data of the treatment of the 33 patients was looked over by a medical physician. Patients were then classified as either a primary or secondary infection according to the new definition of a primary attack. Only 19 of the 33 patients were observed to be a primary infection according to the new definition. Of the patients incorrectly classified by the model only 41% (9/22) were primary infections while 90% (10/11) of those patients correctly classified by the model were primary infections (Table 6.5). An individual's therapeutic response is determined by several factors including baseline parasite burden, degree of immunity, drug levels and drug responsiveness of the infecting parasites. It is likely therefore that these patients were infected by relatively resistant parasites.

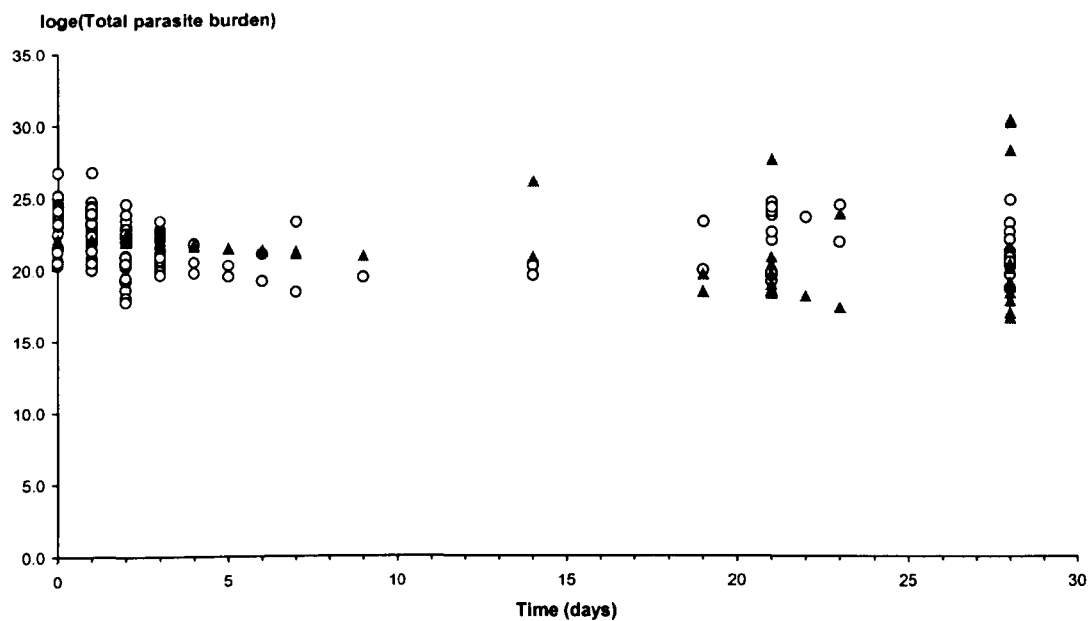
**Table 6.5:- Association between model predictions and demographic and disease related factors.**

Demographic or disease factor	Model predicted failure (correct, n=11)	Model predicted cure (incorrect, n=22)	p-value
Age of patient <sup>a</sup>	14 (0.8, 51)	10 (1.2, 28)	0.418
<5 years	2 (18%)	4 (18%)	
5-14	4 (36%)	11 (50%)	
>14	5 (46%)	7 (32%)	
Temperature on admission <sup>b</sup>	38.4 (0.97)	37.5 (1.02)	0.019
Parasite count on admission <sup>a</sup>	1.1*10 <sup>10</sup> (7.9*10 <sup>8</sup> , 4.2*10 <sup>11</sup> )	1.5*10 <sup>10</sup> (6.7*10 <sup>8</sup> , 8.6*10 <sup>10</sup> )	0.749
Primary infection	10 (90.9%)	9 (40.9%)	0.009

a – median (range); b – mean (SD)

### 6.3.2.3 Sensitivity analyses

Varying the values of the fixed parameters ‘a’ and ‘γ’ did not improve the fit of the model to the data. For example, if the parameter ‘a’ is increased to 1.15 (i.e. a PMR<sub>48</sub> of 10 fold) then the model compensates by reducing  $k_1$  to a very small (unrealistic) number in order to fit the data. The computer programme displays a warning stating that the estimate of  $k_1$  is very close to the lower boundary of the parameter and is probably not interpretable. Figure 6.4 is a scatterplot of the observed and population predicted parasite counts versus time for the model with ‘a’ fixed at 1.15 and ‘γ’ fixed at 2.5. The model predicts that in the first week of infection the total parasite burden hardly changes (estimates  $k_1$ , the rate constant of parasite killing, to be 1.38, that is reducing the parasites approximately 1.6 fold every 48 hours). However the observed parasite counts decrease and drop below the level of detection before reappearing a week or more later.



**Figure 6.4.** Observed parasite counts (○) and population predicted values (▲) versus time for the 33 patients' who failed treatment during 28 days of follow-up (fixed parameters: 'a' = 1.15 and 'γ'=2.5).



## 6.4 Discussion

The mechanistic PD model described in this chapter represents the change in total parasite burden over time and could be used to predict the response of different dosing regimes or for differing areas of resistance. Sequential PK-PD modelling of paired parasite count and mefloquine concentration data has been performed to investigate if the mechanistic PD model fits the observed parasite count data.

### 6.4.1 Summary of findings

The mechanistic PD model did not successfully predict the parasite count data for 22 of the patients. This means that the current model with the five parameters ( $k_1$ ,  $C_{50}$ ,  $P_0$ ,  $a$  and  $\gamma$ ) and mefloquine levels is too simple to predict correctly the response for all patients. Some of the patients were not primary infections, and perhaps were significantly more resistant than the other infections in this series. Thus no model based on PK data and quantifiable PD parameters could ever predict those patients to fail.

Recrudescence could not be distinguished reliably from re-infection because the study took place in an area of malaria transmission. Using data collected from the health clinics in the camps, ter Kuile et al. (1993b) estimated that during the study the mean risk of new symptomatic *P. falciparum* infections was 4.8% (95% confidence interval: 4.4 – 5.2%) per 28 days. If it is assumed that patients are protected for 14 days by prophylactic drug concentrations after treatment with mefloquine then 2.4% (95% confidence interval: 2.1 – 2.7%) of the study patients would have a new symptomatic infection during the 28-day followup period. In other words it is possible that 4 of the 33 patients classified as a recrudescence were actually harbouring a new infection.

The results from the sequential PK-PD modelling did give realistic population mean estimates for the parameters,  $k_1$ ,  $C_{50}$  and  $P_0$ . White et al. (1997) proposed that the killing action of mefloquine was between 10 & 1000 fold every 48 hours and Price et al. (1999a) proposed that the MIC *in vivo* of mefloquine was approximately 500 ng/ml. From the modelling of the current data  $k_1$  was estimated to be 80 fold every 48 hours and the MIC *in vivo* to be 562 ng/ml.

#### 6.4.2 Limitations of sequential pharmacokinetic-pharmacodynamic modelling

Sequential population PK-PD modelling is relatively new and there are problems associated with the method. To model successfully the response, a reasonable range of concentrations across subjects is required. It is also important to choose the appropriate response for the PD model. In other words the clinical relevance, ease of measurement, variability in the response and overall sensitivity to changes in drug concentration need to be considered.

Instead of using sequential PK-PD modelling, simultaneous PK and PD modelling (see section 1.2.3.2 for more details) could have been applied to the data. General opinion is divided between whether it is more informative to perform a simultaneous or sequential analysis. Despite this debate, it is well established that the presence of PK data (even small amounts of suboptimally sampled PK data) improves the precision of the estimates of the PD parameter values (Hashimoto and Sheiner, 1991).

#### 6.4.3. Limitations of the model

The PD model presented in this chapter does not include any parameters to represent the stage-specific killing action of mefloquine. However a lag phase of one day was included to account for the stage-specificity of mefloquine. The antimalarial mefloquine has a long half-life and therefore it may not be so important to include in the model the stage-

specificity of the drug. Conversely for a drug with a short half-life such as artesunate the stage dependency of the drug is important. Svensson (2000) used the life cycle of the *P. falciparum* parasite as the basis for a mechanistic model to address the PD effect of artemisinin, mefloquine and the combination. The model consisted of four parasite compartments representing different asexual parasite stages (ring stages, early trophozoites, late trophozoites and schizonts). Models in which mefloquine acted on a different number of parasite compartments did not describe the data better than a model in which mefloquine acted on all four parasite compartments.

The model also does not include a parameter to represent immunity. However the 22 patients incorrectly predicted by the model had a similar age distribution to the eleven patients correctly predicted (see Table 6.5).

#### 6.4.4 Limitations of the data

The observed data is limited to the relatively narrow range of detectable parasites (i.e.  $> 2.5 \times 10^8$  parasites). This means there is little information for estimating the concentration where 50% of the parasites are killed because at these concentrations the number of parasites in the blood cannot be detected. Any parasite counts recorded as zero (i.e. below the level of detection) were excluded from the analyses. Whether to exclude these values or set them to the limit of determination is much debated in the field of pharmacokinetics. Rahman et al. (1999) examined the effect of deleting time points at which the drug concentration was recorded as below the limit of quantification, as opposed to including these points as censored observations. They observed that including the censored data results in greater precision of the PK parameters. Additionally the parameter representing the final slope of the curve becomes steeper when the censored data are included giving rise to smaller half-lives of the drug. The model fitted to the data was a 3-compartment model, which is an exponentially decreasing function. The model presented in this chapter

is a parabola and the parasite counts below the level of detection occur around the minimum of the parabola. Thus exclusion of these data points may have an even greater impact on the parameter estimates besides the reduced precision identified by Rahman et al. (1999).

Another problem regarding the observed parasite count data is that the observed data only represents the parasitised red blood cells in the peripheral blood (not the sequestering parasites). Depending on the distribution of the age of the parasites, the number of parasites in the peripheral blood may represent only a fraction of the total parasite burden. More severe infections have been thought to be related more to the sequestering parasites than to the younger stages circulating in the blood (White, 1998).

## **7. SUMMARY AND IMPLICATIONS FOR FUTURE RESEARCH.**

Despite the high rates of mortality and morbidity associated with malaria very few new antimalarial drugs are being developed (Trouiller and Olliaro, 1999). Hence there is much to be gained by optimising currently available antimalarials. Mefloquine is the most widely used drug for the treatment of multidrug-resistant *falciparum* malaria. Using pharmacokinetic (PK) and pharmacodynamic (PD) data from field trials, where mefloquine was administered either alone or in combination with artesunate, important information regarding dosing for the clinician has been obtained. Moreover a mechanistic PD model has been derived which can be used as a prediction tool for therapeutic efficacy of mefloquine for differing dosages and differing areas of resistance. PK, PD (*in vivo* and *in vitro*) and malaria therapy data have been modelled to obtain estimates of the parameters in the mechanistic PD model.

### **7.1 Population pharmacokinetics of mefloquine.**

In Chapter 3 a retrospective population PK analysis of mefloquine data from patients receiving mefloquine as part of trials conducted between 1990 and 1995 was performed. The population PK analysis was performed to provide information that would help to optimise the dosage of mefloquine, both when used alone and in combination with an artemisinin derivative.

The study population consisted of 257 patients treated for uncomplicated *P. falciparum* in malaria clinics situated on the western border of Thailand. There were four different treatment groups; mefloquine monotherapy (25 mg/kg (single dose) or 25 mg/kg (split dose: 15 mg/kg on day 0 followed by 10 mg/kg 24 hours later)) and combined therapy of

mefloquine and artesunate (mefloquine given as either a single dose or a split dose). In total 432 whole blood mefloquine concentrations were available for data analysis from the patients receiving mefloquine monotherapy, ranging between 1 and 7 per individual (median 3). This data set was generally “sparse” as 50% of the patients had 3 or less blood samples recorded. For the patients receiving combined therapy 588 whole blood mefloquine concentrations were available, ranging between 3 and 7 per individual (median 6).

The mefloquine PK were influenced relatively little, or not at all, by factors such as age, sex, or measures of acute malaria severity. Splitting the 25 mg/kg dose of mefloquine monotherapy was associated with a 50% increase in oral bioavailability, and bioavailability increased by 20% for the split dose on the combination treatment. Oral bioavailability was also observed to be associated with more rapid parasite clearance, a surrogate outcome measure for cure. The apparent volume of distribution (V/F) was significantly lower in patients’ receiving split doses of mefloquine monotherapy (mean 8.14 l/kg; 95% CI: 7.49 to 8.86 l/kg) compared with a single dose (mean 20.37 l/kg; 95% CI: 16.26 to 25.51 l/kg). If it is assumed that apparent clearance and volume of distribution are unaffected by dose regimen, then splitting the 25 mg/kg mefloquine dose improves oral bioavailability and the therapeutic response in the treatment of acute falciparum malaria.

## **7.2 Mefloquine pharmacokinetic-pharmacodynamic model.**

In Chapter 4 a mechanistic mathematical model was developed that describes the change in total parasite burden with time *in vivo*, in the presence of mefloquine. The model consists of seven parameters: two of which represent the PK of mefloquine, and another two PD parameters that represent the mefloquine concentration effect curve. Estimates for these PK and PD parameters were obtained from population modelling of PK and *in vitro* PD

data from the same region (i.e. western border of Thailand) respectively. The other three parameters: parasite multiplication rate, total parasite burden on admission, and mefloquine parasite killing rate, were estimated using values from the literature.

Model simulations confirmed that, early in the evolution of resistance, conventional assessments of the therapeutic response 28 days after treatment underestimate considerably the level of resistance. Longer follow-up is required. The model also indicates that initial deployment of a lower (15-mg/kg) dose of mefloquine provides a greater opportunity for the selection of resistant mutants and would be expected to lead more rapidly to resistance than *de novo* use of the higher (25-mg/kg) dose.

### **7.3 Population dynamics *in vivo* of the natural infection of falciparum malaria.**

In Chapter 5 estimates were obtained for the parasite multiplication rate every 48 hours and periodicity of the parasite cycle from modelling of a unique dataset. In the early part of the 20<sup>th</sup> century, before the introduction of penicillin, patients suffering from neurosyphilis in Europe and the United States were treated by inducing a protracted attack of falciparum or vivax malaria. The number of parasites within a patient was recorded daily, and these records provide a unique source of information regarding the natural history of malaria in the non-immune adult host. The statistical modelling of the data was restricted to the first week of patent parasitaemia, the period of growth when host defences have minimal effect.

The patient parasitaemia versus time profiles varied greatly between patients. Parasite multiplication rate every 48 hours was estimated to be approximately 8 fold with a 95% prediction interval of 5.5 up to 12.3 fold. The parasite life cycle was estimated to be 55 hours, however this estimate is highly dependent on the sampling interval of one day.

## 7.4 Sequential pharmacokinetic-pharmacodynamic modelling for mefloquine

Sequential PK-PD modelling was performed in Chapter 6 on paired parasite count and mefloquine concentration data collected from 33 patients whom recrudesced (RI resistance) following treatment with mefloquine monotherapy. A patient suffering from *P. falciparum* can recruduesce due to a number of factors including the killing rate, absorption and plasma half-life of the drug, and the drug susceptibility of the parasite. The mechanism based PD model used for the sequential PK-PD modelling included parameters for the killing rate and PK of the drug and successfully fitted 11 of the patients. The remaining 22 patients were predicted by the model to be radically cured. Thirteen of these patients did not have primary infections and therefore could contain parasites that are highly resistant to mefloquine, a difficult confounder to measure and include in the model. Approximately four of these patients could have new infections as opposed to recrudescences. Techniques now exist to differentiate between treatment failures and new infections but these were not available during 1990 and 1991, the years when the parasite count and mefloquine concentration data were collected.

Although the current mechanistic PD model did not successfully fit 66% of the patients it is one of the few attempts where a mechanistic PD model for mefloquine has been sequentially fitted to parasite count and mefloquine concentration data. Reasons for the poor fitting could be: recrudescences actually being new infections, secondary infections (i.e. retreated infection), major sequestering of the parasites in the first week of patent parasitaemia, sparseness of the observed parasite count data, model misspecification and finally logistic reasons, that is microscopic level of detection.



## 7.5 Further issues and future research

The development of population PK-PD models provides a framework for optimisation of antimalarial dosing regimens. The modelling presented in this thesis provides a good start for future PK-PD models. However there are two main areas that need to be discussed and researched further.

- 1) Does the mechanistic PD model developed in this thesis need to be extended to include more parameters describing the intra-host malaria dynamics before it can inform clinicians regarding optimal dosing and the development of drug resistance? (section 7.5.1)
- 2) Can the approach to PK-PD model development for mefloquine in this thesis be utilised for development of PK-PD models of other antimalarial treatments? (section 7.5.2)

The PK and PD parameters of mechanistic PD models need to be estimated from PK and PD data. Besides retrospective modelling of datasets currently available, new data need to be collected with the aim of using the data for population PK-PD modelling. With the collection of more detailed parasitaemia data, the mechanistic PD model presented in this thesis could be expanded to analyse parasite age structure more accurately. Guidelines regarding the design of population PK and PD studies are presented in section 7.5.3.

### 7.5.1 Current mechanistic pharmacodynamic model requires more parameters?

The mechanistic PD model described in this thesis did not adequately describe all 33 patients who recrudesced within 28 days. Many reasons for this lack of fit have been

discussed in Chapter 6. For example, the model needs more parameters to describe the *in vivo* parasite versus time relationship. Currently the model has only one parameter that describes the change in parasite burden over time in the absence of drug, that is the parasite multiplication rate. Other factors that change the parasite burden with time include host-immunity (both non-specific and specific), synchronicity of the parasite distribution, and sequestration of the parasites.

#### 7.5.1.1 Host immunity

The natural immunity of a patient can affect the dynamics of the *P. falciparum* infection. The mechanistic model (equation 6.1, Chapter 6) could be extended to include a function that represented the immunity of a patient (see below).

$$\frac{dP_{ij}}{dt} = a.P_{ij} - [k_1 \left( \frac{C_{ij}^{\gamma}}{C_{ij}^{\gamma} + C_{s0}^{\gamma}} \right)].P_{ij} - f(I)P_{ij}$$

where  $f(I)$  is a function representing host immunity.

Three possible measures to represent immunity that are easily obtainable from patient data are the age of the patient, the temperature of the patient at each parasite recording and the size of the patient's spleen. Another measure to represent host immunity is the host's previous exposure to malaria, also obviously a function of age, however this is highly unreliable due to recall bias. Bruce et al. (2000) observed that infections often last for several weeks in young children but generally for only a few days in adults. This variation is widely held to be a result of acquired immunity, resulting from multiple infections. Consequently the age of the patient could be used as a surrogate measure for natural immunity. Note the level and variation of immunity across different age groups will differ for areas of high and low transmission of *P. falciparum*.

Another measure investigated to represent non-specific host immune defences was the presence of fever (Kwiatkowski and Nowak, 1991; Gravenor and Kwiatkowski, 1998a). Fever was found to be the main regulatory factor of parasite burden (in the absence of drug) for the early part of the infection. The models investigated the early control of *P. falciparum* density through a toxic feedback system. Once the parasite rose above a value called the “pyrogenic threshold”, fever would develop and suppress parasite growth. However it is difficult to assign a single level to represent the pyrogenic threshold of all patients. Jeffery et al. (1959) demonstrated that malaria therapy patients vary by orders of magnitude in the density at which they control their parasitaemia and in the parasite density associated with first fever. The pyrogenic threshold of the patient may be a better measure of immunity. The patient data modelled in this thesis consisted of symptomatic patients. Thus their pyrogenic threshold is equal to or below the parasitaemia recorded on admission; a parameter already included in the mechanistic PD model.

Spleen size is sometimes used as a marker of endemicity (Gilles, 1993) and therefore is linked to immunity. It usually reflects previous contact with malaria and non-specific immunity. During a *P. falciparum* infection the spleen within a patient rapidly enlarges to clear the parasites from the circulating red cells (Angus et al. 1997; Chotivanich et al. 2000b).

All of the measures mentioned above represent non-specific measures of host immunity. Specific measures of host immunity such as antibodies and cell mediated responses are very difficult to quantify and measure especially when the patient has a new active infection.

### 7.5.1.2 Synchronicity

The age of the *P. falciparum* parasites range from 0 to 48 hours. The term ‘synchronicity’ represents the spread of the age of the parasites in a single infection. A highly synchronous infection means the age of the parasites within the patient are very similar whereas an asynchronous infection represents a patient harbouring parasites of all ages. Infections that are synchronous will have periodic peripheral parasite versus time profiles. However asynchronous infections will have non-fluctuating parasite-time profiles. White et al. (1992a) suggested a simple mathematical model to describe the changes in circulating and sequestered parasite numbers with time. The model uses two parameters only; the standard deviation of parasite age as a measure of synchronicity, and a multiplication factor each 48 hour asexual life cycle. Hoshen et al. (2000a) combined the two methods of White et al. (1992a) and Anderson et al. (1989) to form a single model that explains the periodicity of malaria and includes immunity and chemotherapy. In both of the above studies parasite-time profiles were simulated and compared visually to observed patient data. No attempt was made to estimate the parameters from the data using statistical modelling techniques. Svensson (2000) included in their model a parameter that represented the time for one life cycle in the average parasite, that is parasite mean residence time. They stated that by varying this parameter the parasites are allowed to distribute over the four compartments (ring stages, early trophozoites, late trophozoites and schizonts) during the two life cycles before the introduction of drug into the system. Individuals with parasites that have similar residence times are highly synchronised. This will account for the possibility of infections being more synchronised in some patients compared to others. Although the parameter does not measure the synchronicity of an infection it does present a way of taking account of the variation across patients resulting from synchronicity. Unfortunately the data did not support estimation of parasite mean residence time and the corresponding inter-parasite variability. Instead parasite mean residence time was fixed at 48 hours.

The synchronicity of an infection varies markedly across patients. In Chapter 5 some of the malaria therapy patients had periodic parasitaemia-time profiles whereas some had profiles that did not oscillate. For the empirical model fitted to the malaria therapy patients the amplitude of the sine wave of equation 5.2 could be considered to empirically represent the synchronicity of an infection. Patients with very large amplitudes have highly synchronous infections whereas moderately synchronous infections have amplitudes close to zero. Non fluctuating patient parasite profiles could not be fitted by equation 5.2 because there was no periodicity in the profiles.

*In vivo* it is not possible to measure the synchronicity of an infection as not all parasite stages are present in the peripheral blood. Therefore to calculate the total parasite burden and the number of parasites within each stage one needs to extrapolate from data collected which describes the distribution of parasite stages in the peripheral blood. Retrospective investigation of blood films from severe and non-severe infections confirmed that a predominance of mature parasites in the peripheral blood reflects a greater sequestered biomass, and thus more severe disease (Silamut and White, 1993). Staging of intra-dermal smears has also been performed (Nakazawa et al. 1995). In the intra-dermal smears later stages of the parasite have been observed and this smear may therefore be a better representation of the distribution of all parasites. However staging of both peripheral blood and intra-dermal smears still only represents proxy distributions of the parasite age. Even with these proxy distributions of parasite age it is still difficult to mechanistically quantify synchronicity. The proposed model presented by Svensson (2000) did not give a measure of synchronicity but did take into account the synchronicity of an infection using a measure of parasite mean residence time. Further research involving data that includes some knowledge of the parasite distribution (i.e. peripheral parasite count data where staging has been performed) is required to fully quantify the models based on the parasite's life cycle.

### 7.5.1.3 Sequestration

One of the major problems associated with modelling of falciparum malaria is that the *P. falciparum* parasite disappears from the blood after about 24 - 26 hours. This process is known as sequestration and accounts for the discrepancy between peripheral blood parasitaemia (microscopically visible) and total parasite burden. Clearly it is difficult to form a reliable picture of the response to antimalarial therapy without knowing the behaviour of the sequestered parasite population. Unfortunately there is no direct method of measuring the sequestered parasites. Gravenor et al. (1998b) developed an age-structured mathematical model with two compartments (circulating and sequestering parasites) that allows the number of sequestered parasites to be estimated from sequential observations of parasitaemia on peripheral blood smears. Their results suggest that peripheral parasitaemia can predict clinical outcome (e.g. recrudescence), but only insofar as it reflects the number of sequestered parasites. This is not the case in highly synchronous infections or during treatment with an antimalarial drug that acts on a specific stage of parasite development. Note the patient data used in the modelling was from severe malaria patients treated with the short acting drugs, artesunate and quinine. The model developed in this thesis was for mefloquine, a drug with a long-half life used for treating patients with uncomplicated malaria.

Often mechanistic models are criticised for not having parameters describing every feature of the disease. However a model cannot encompass every feature, but hopefully the missing features are not crucial (McKenzie, 2000). The value of a mathematical model is its ability to identify what factors are important and need to be considered when deciding on drug policies. The model suggests that the evolution of resistance can be substantially delayed by the appropriate choice of drug treatment regimen (Hastings and D'Alessandro, 2000).

### 7.5.2 Application of population pharmacokinetic-pharmacodynamic modelling to other antimalarials

Population PK modelling has been applied to lumefantrine (Ezzet et al. 2000), artemisinin (Sidhu et al. 1998), proguanil (Hussein et al. 1996), atovaquone (Hussein et al. 1997), and both mefloquine and artemisinin (Svensson 2000). For lumefantrine the prospective population PK-PD study found the area under the concentration-time curve (AUC) to be the principle determinant of antimalarial activity. For artemisinin, Sidhu et al. (1998) failed to identify any associations between the PK and PD parameters. For proguanil and atovaquone, retrospective population PK analyses of data from Phase II/III clinical trials were performed to examine the effect of patient covariates and dose. Svensson (2000) performed population PK modelling to investigate whether artemisinin influences the PK of RS- and SR- enantiomer of mefloquine. Further, a PD parasite model was developed, based on the parasite's life cycle describing the efficacy of artemisinin, mefloquine and a combination of the two drugs.

Mechanistic PD models have been developed for chloroquine (Hoshen et al. 1998), artesunate (Hoshen et al. 2000b), and both mefloquine and artemisinin (Svensson 2000). For both chloroquine and artesunate, simulations for different dosages, etc. were presented but no formal fitting of the model to PK-PD data was performed. The mefloquine PD model presented in this thesis was an extension of the chloroquine PD model including a parameter for the slope of the concentration-effect curve. The model for artesunate included stage specificity of the drug action since artesunate has a very short half-life. It was observed that standard PK-PD modelling failed to explain the clinical results and lead to the postulation that a small fraction of the parasites, as a result of chemotherapeutic pressure, become cytostatic or 'dormant'. Svensson (2000) developed a mechanistic PD parasite model that was based on the parasite's life cycle (defined as 4 stages: ring stages, early trophozoites, late trophozoites and schizonts), and the PK of the antimalarial drugs.

The PD parameters were estimated using population sequential PK-PD modelling of the patient parasite density-time profiles. The mechanism-based PD model successfully predicted the antimalarial effect of artemisinin, mefloquine and a combination of the two drugs.

The mechanistic PD model developed in this thesis could be adapted for application to other antimalarial treatments. It is a simple model where the essential parameters can be estimated from clinical and laboratory data. Two major factors need to be considered when adapting the model for other antimalarial drugs: the plasma half-life and stage specific action of the antimalarial. Antimalarial drugs vary greatly in both stage-specificity (ter Kuile et al. 1993a; Geary et al. 1989) and plasma half-life (White, 1992c) and both of these factors can have major effects on the parasite clearance (White, 1997). Once the model has been formulated, formal testing (i.e. sequential PK-PD modelling) and validation are required (Machado et al. 1999). The mechanistic PD model could then be used to simulate important clinical scenarios to inform the clinician on the optimal dosage required for their patients with *P. falciparum*.

Population PK-PD modelling has been performed successfully for drugs that treat other medical conditions. Some examples are the drugs: ivabradine (Duffull and Aarons, 2000), ketorolac analgesia (Mandema and Stanski, 1996), theophylline (Holford et al. 1993) and levodopa (Triggs et al. 1996). For the drug ivabradine, a novel bradycardic agent that has been developed for the prevention of angina, a sequential linked PK and PD simulation model was developed. It is intended to use this model as the basis for designing clinical trials where exercise tests are the intervention for measurement of the outcomes. For ketorolac analgesia, the treatment of moderate to severe postoperative pain, the derived population PK-PD model simulated the relationship between dose, time, and the percentage of patients with adequate pain relief. The simulations suggested that 30 mg



intramuscular ketorolac was the optimal initial dose for postoperative pain relief. For theophylline, a treatment for episodes of severe airways obstruction, the PK-PD modelling observed that the action of theophylline was most marked at the start of treatment, theophylline may no longer have important beneficial effects after 72 hours, and the incidence of adverse effects increased at theophylline concentrations  $>20$  mg/l. For levodopa, the principle treatment for symptomatic control of Parkinson's disease, the PK-PD model was observed to have satisfactory predictive performance and may in the future prove valuable as a means for optimising individual patient dosage schedules.

### 7.5.3 Study design for population pharmacokinetic-pharmacodynamic studies of antimalarials

For new antimalarial drugs it is vital that within the design of the Phase III trial data are collected for population PK-PD modelling either from a sub-group of patients or from each patient. For current antimalarial drugs, field trials investigating the efficacy of the drug need to allow for collection of PK-PD data. Moreover *in vitro* data should be collected to provide information about drug resistance.

Prospective population PK-PD studies have been performed for the antimalarial drugs, lumefantrine (Ezzet et al. 2000) and artemisinin (Sidhu et al. 1998). For lumefantrine, data were combined from a conventional inpatient PK study of adults and a population based community study of patients of all ages. The objective of the study was to identify the factors that affect the PK of lumefantrine and thus the therapeutic response. The study population consisted of 51 adult patients treated at a hospital in Bangkok, Thailand, and 215 patients aged more than two years treated at a malaria clinic in Mae La on the western border of Thailand. A total of 655 venous samples were available from the 51 patients in Bangkok, approximately 14 samples per patient. For the 215 patients from Mae La 793 capillary samples were available, averaging four samples per patient. All patients were

seen daily for the first 5 days and then weekly from day 7 to day 63 for recording of parasite count, temperature, subjective adverse effects and a neurological examination.

Sidhu et al. (1998) performed a prospective study of artemisinin where patients were allocated randomly to two blood sampling occasions on day 1 of treatment and where a subgroup of patients had a single blood sample taken on day 5. The sampling schemes were assessed before the study by using data obtained from a previous study of the PK of oral artemisinin in adult patients where many blood samples per patient were collected (12 samples from each of 15 subjects). The motivation to perform a prospective population PK study of artemisinin was the lack of PK knowledge in the paediatric population because of the problems associated with performing intensive blood sampling and limitations in sensitive analytical methodologies. The study population consisted of 23 children (aged 2 to 12 years) and 31 adults (aged 16 to 45 years) living in Song Be Province, Vietnam. A total of 140 concentrations (107 concentrations on day 1 of treatment, 33 concentrations on day 5) were available from 54 patients with acute, uncomplicated *P. falciparum* malaria. Blood samples were collected on day 5 to investigate the inter-occasion variability in the PK parameters since constancy over time in the PK of artemisinin was unlikely to be a valid assumption. The parasitaemia measurements were timed to coincide with blood sampling for the PK determinants to investigate the association between the PK of artemisinin and the PD outcome measures.

Population PK-PD studies can form part of large clinical trials where a few blood samples are taken from each patient or from a randomly selected sub-group of patients. The methods for the population PK-PD study must be part of the protocol. Recommendations regarding the design of population PK-PD studies are listed below:-

- 1) To determine the timing of blood samples and the number of subjects, computer simulation and optimal design methods (D'Argenio, 1981; Mentré et al. 1997; Retout et al. 2001) can be used. These methods ensure that data are collected at informative times for estimation of the PK-PD profiles. Note designs involving more individuals, even if many are sparsely sampled, dominate designs calling for many samples on fewer persons (Hashimoto and Sheiner, 1991).
- 2) Patients should be sampled throughout the duration of the study to allow for changes over time in the PK parameters (known as inter-occasion variability).
- 3) The study investigator should be given a sampling window (that is, a range of times rather than a particular time) when a blood sample must be collected. It is important that the investigator understands the importance of accurately recording dosing and sampling times.
- 4) The times of blood sampling should be convenient both to patients and to clinical staff.

## **7.6 Conclusion**

The development of resistance to antimalarial drugs is a major threat to the health of people living in tropical countries. The speed at which resistance develops is affected considerably by the pattern of antimalarial drug use. When new antimalarial drugs are introduced, dose-ranging studies are performed to determine the optimum dose and treatment schedule on the basis of the therapeutic ratio. The prevention of resistance is usually not considered in this assessment. With a mechanistic PD mathematical model a more effective method exists to inform clinicians regarding drug usage and the

development of drug resistance. Accurate predictions from the model depend on precise estimates of the PK, PD and parasite growth parameters. In this thesis estimates of these parameters have been obtained for mefloquine from population modelling of PK, PD *in vivo*, PD *in vitro* and historical malaria therapy datasets.

Modern malaria research requires mathematicians and statisticians working alongside clinicians and economists. With this multidisciplinary group the costs of resistance in terms of both morbidity and perhaps mortality need to be balanced against the cost of an antimalarial drug to ensure that the small financial budgets of developing countries are used effectively for the current and future burden of malaria.

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## APPENDIX I

SPLUS programme for fitting a one compartment model.

Dosing regime – 15 mg/kg at time 0 followed by 10 mg/kg 24 hours later.

```
# fitting mefloquine with a 1 compartment model (oral dosage)
compl.oral<-
function(cl,v,DOSE,TIME)
{
  ((7*DOSE)/((v*7)-cl))*(exp(-(cl/v)*TIME)-exp(-(7*TIME)))
}

all.nlme<-
function()
{nlme(CONC ~
  (Q1*compl.oral(cl*exp(eta.cl),v*exp(eta.v),1000*DOSE1,T1)+
    Q2*compl.oral(cl*exp(eta.cl),v*exp(eta.v),1000*DOSE2,T2)
  ),
  data = pkvensp.comb,
  fixed = list(cl~.,v~.),
  random = list(eta.cl~.,eta.v~.),
  start = list(fixed = c(0.6,20)),
  cluster = ~ID,
  re.structure = "d",
  var.function = "identity",
  verbose=T
)
}
```

## APPENDIX II

SPLUS programme for fitting a one compartment model.

Dosing regime – 25 mg/kg at time 0

```
# fitting mefloquine with a 1 compartment model(oral dosage)
compl.oral<-
function(cl,v,DOSE,TIME)
{
  ((7*DOSE*1000)/((v*7)-cl))*(exp(-(cl/v)*TIME)-exp(-(7*TIME)))
}

all.nlme<-
function()
{nlme(CONC ~
  compl.oral(cl*exp(eta.cl),v*exp(eta.v),DOSE,TIME),
  data = pkvensi.comb,
  fixed = list(cl~.,v~.),
  random = list(eta.cl~.,eta.v~.),
  start = list(fixed = c(0.6,20)),
  cluster = ~ID,
  re.structure = "d",
  var.function = "identity",
  verbose=T
)
}
```

### APPENDIX III

To find  $P_t$  integrate  $\frac{dP}{dt}$  :-

$$\frac{dP}{dt} = a.P - f(C).P$$

substitute  $f(C) = k_1 * \frac{C^\gamma}{C^\gamma + C_{50}^\gamma}$

thus

$$\frac{dP}{dt} = \left[ a - k_1 * \left( \frac{C^\gamma}{C^\gamma + C_{50}^\gamma} \right) \right] * P$$

$$\frac{dP}{dt} = f(t) * P$$

[since  $C$  is a function of time and  $a, k_1, \gamma$  &  $C_{50}$  are constants]

Using the separation of variables method to integrate:-

$$P_t = P_0 * e^{\int f(t) dt}$$

$$\int f(t) dt = a.t - k_1 * \int \frac{C^\gamma}{C^\gamma + C_{50}^\gamma} dt$$

where

$$\int \frac{C^\gamma}{C^\gamma + C_{50}^\gamma} dt = \int \frac{(C_0 * e^{-k.t})^\gamma}{((C_0 * e^{-k.t})^\gamma + C_{50}^\gamma)} dt$$

$$= \frac{-1}{k \cdot \gamma} * \left[ \log_e \left( \frac{C_{50}^\gamma + (C_0 * e^{-k.t})^\gamma}{C_{50}^\gamma + C_0^\gamma} \right) \right]$$

$$P = P_0 * e^{a.t} * \left[ \frac{C_{50}^\gamma + (C_0 * e^{-k.t})^\gamma}{C_{50}^\gamma + C_0^\gamma} \right]^{k_1 / (k \cdot \gamma)}$$



## APPENDIX IV

SPLUS programme for fitting an inhibitory sigmoid  $E_{\max}$  effect model.

```
# fitting concentration-effect curve to mefloquine
compl.invitro<-
function(a,b,ic,g,M.CONC)
{
  (b - (b-a)*((M.CONC^g)/((M.CONC^g) + ((ic^g)/9))))
}

all.nlme<-
function()
{nlme(EFF.PERE ~
      compl.invitro(a,b*exp(eta.b),ic*exp(eta.ic),g*exp(eta.g),M
        .CONC),
  data = merge.thai,
  fixed = list(a~.,b~.,ic~.,g~.),
  random = list(eta.b~.,eta.ic~.,eta.g~.),
  start = list(fixed = c(1,100,50,3)),
  cluster = ~ID,
  re.structure = "d",
  var.function = "identity",
  verbose=T
)
}
```

## APPENDIX V

SPLUS programme for fitting the empirical model to the patient data from studies conducted between 1931 and 1963 where malaria was used for the treatment of neurosyphilis.

```
# Fitting a function to the patient data
model.vol<-
function(a,b,c,d,k,DAY)
{
    a + (0.5*log10(b)*DAY) + (c*sin((6.28/d)*DAY + k))
}

all.nlme<-
function()
{nlme(LG10PARAM ~
    model.vol(a+(eta.a),b*exp(eta.b),c*exp(eta.c),d,k,DAY),
    data = nlme.146,
    fixed = list(a~.,b~EL.LIMON+SANTEE.C,c~.,d~.,k~.),
    random = list(eta.a~.,eta.b~.,eta.c~.),
    start = list(fixed=c(1.3,8.2,0,0,0.5,2.4,0.4)),
    cluster = ~PATIENT,
    re.structure = "d",
    var.function = "identity",
    na.action = na.omit,
    verbose = T
    )
}
```

## APPENDIX VI

Publications arising from this thesis:-

**Simpson, J. A.,** Price, R., ter Kuile, F., Teja-Isavatharm, P., Nosten, F., Chongsuphajaisiddhi, T., Looareesuwan, S., Aarons, L., White, N. J. (1999) Population pharmacokinetics of mefloquine in patients with acute falciparum malaria. *Clinical Pharmacology and Therapeutics*, **66(5)**, 472-484.

**Simpson, J. A.,** Watkins, E. R., Price, R. N., Aarons, L., Kyle, D. E., White, N. J. (2000) Mefloquine pharmacokinetic-pharmacodynamic models: implications for dosing and resistance. *Antimicrobial Agents and Chemotherapy*, **44(12)**, 3414-3424.

**Simpson, J. A.,** Aarons, L., White, N. J. (2001) How can we do pharmacokinetic studies in the tropics? *Transactions of the Royal Society of Tropical Medicine and Hygiene* In press.